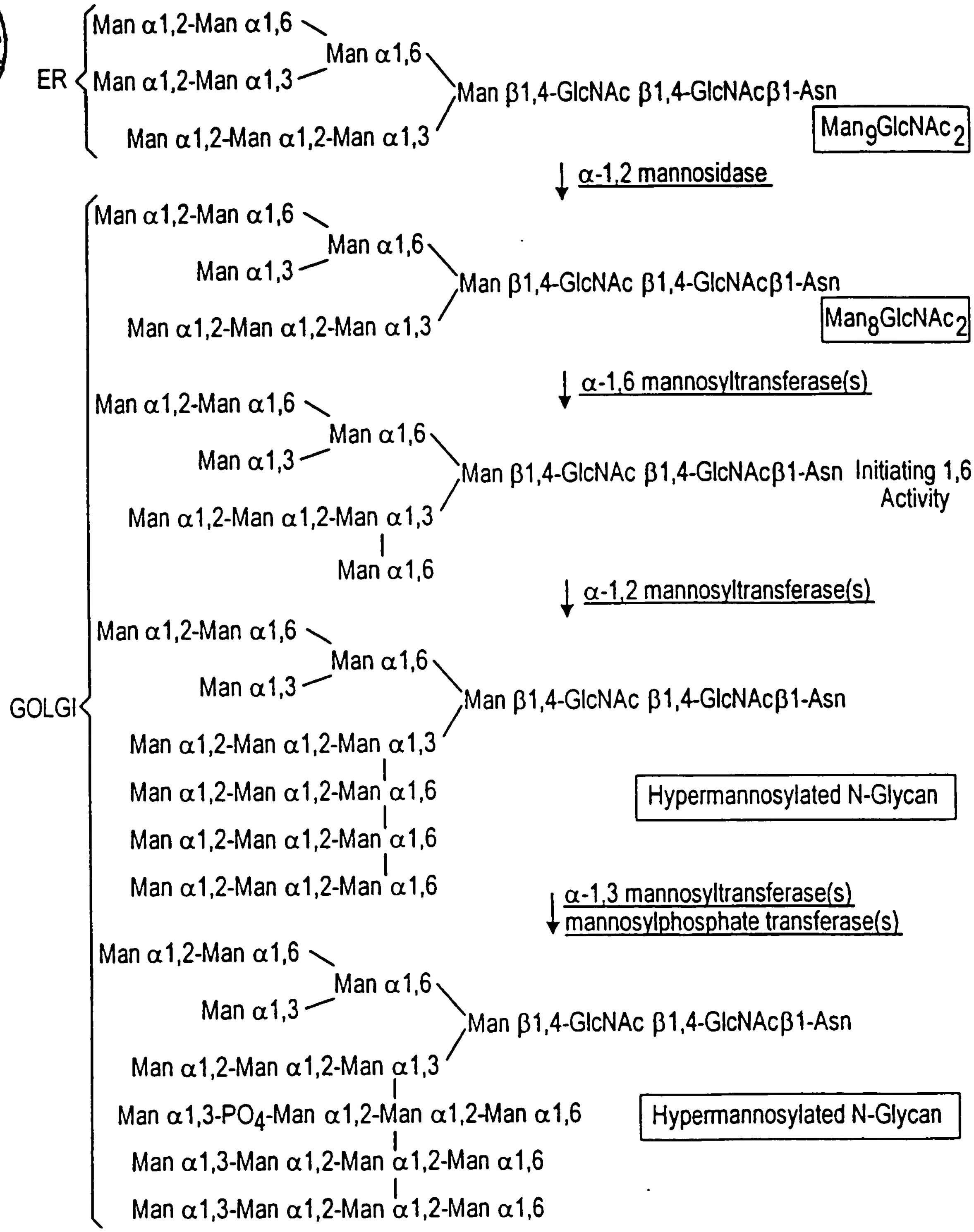

ER
Man α1,2-Man α1,6
Man α1,6
Man α1,2-Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,2-Man α1,2-Man α1,3
$Man_9GlcNAc_2$
α-1,2 mannosidase
Man α1,2-Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,2-Man α1,2-Man α1,3
$Man_8GlcNAc_2$
α-1,6 mannosyltransferase(s)
Man α1,2-Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Initiating 1,6 Activity
Man α1,2-Man α1,2-Man α1,3
Man α1,6
α-1,2 mannosyltransferase(s)
GOLGI
Man α1,2-Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,2-Man α1,2-Man α1,3
Man α1,2-Man α1,2-Man α1,6
Man α1,2-Man α1,2-Man α1,6
Man α1,2-Man α1,2-Man α1,6
Hypermannosylated N-Glycan
α-1,3 mannosyltransferase(s)
mannosylphosphate transferase(s)
Man α1,2-Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,2-Man α1,2-Man α1,3
Man α1,3-PO_4-Man α1,2-Man α1,2-Man α1,6
Man α1,3-Man α1,2-Man α1,2-Man α1,6
Man α1,3-Man α1,2-Man α1,2-Man α1,6
Hypermannosylated N-Glycan

FIG. 1A

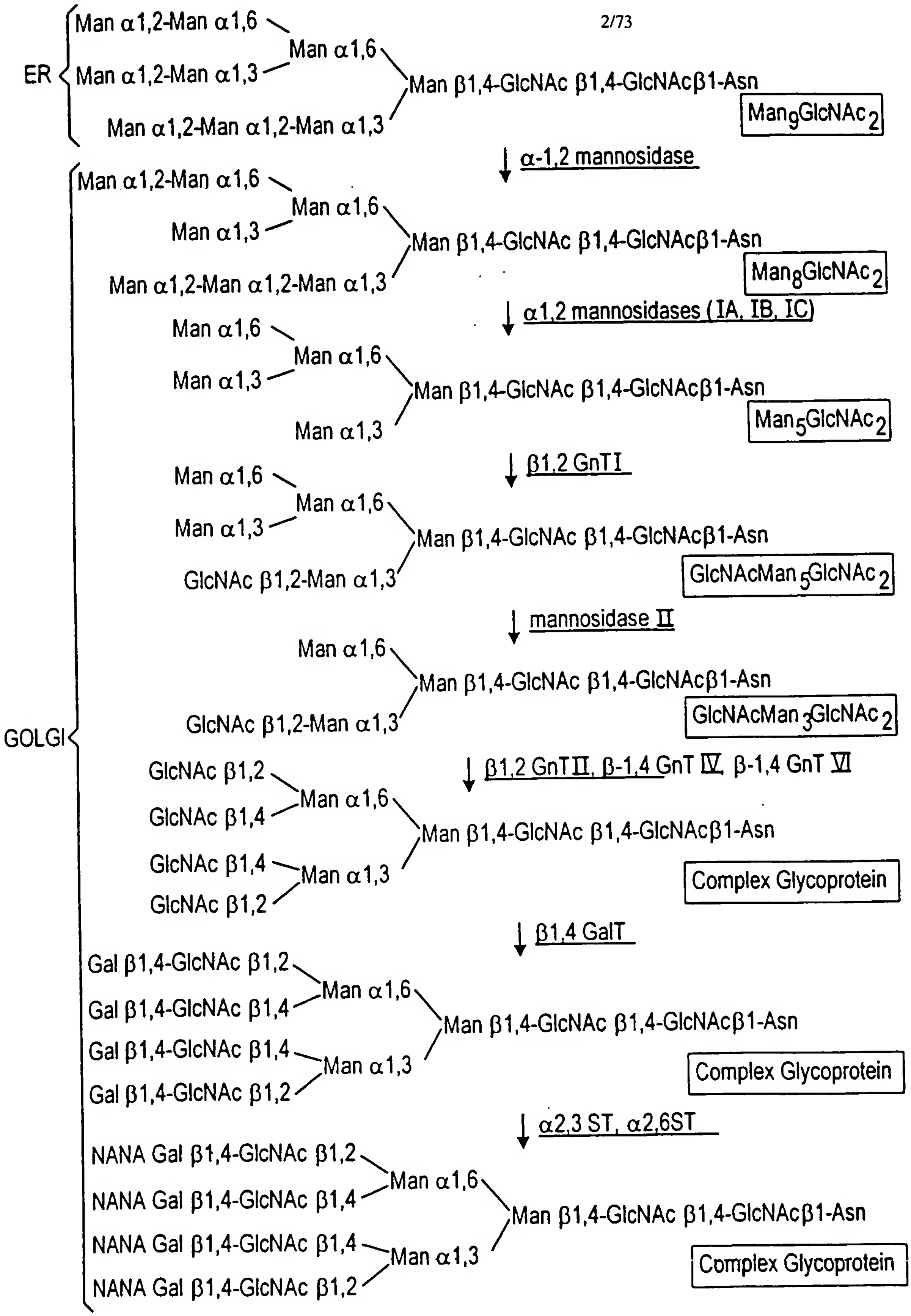

ER
Man α1,2-Man α1,6
Man α1,6
Man α1,2-Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,2-Man α1,2-Man α1,3
Man9GlcNAc2
α-1,2 mannosidase
Man α1,2-Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,2-Man α1,2-Man α1,3
Man8GlcNAc2
α1,2 mannosidases (IA, IB, IC)
Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Man α1,3
Man5GlcNAc2
β1,2 GnT I
Man α1,6
Man α1,6
Man α1,3
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
GlcNAc β1,2-Man α1,3
GlcNAcMan5GlcNAc2
mannosidase II
Man α1,6
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
GlcNAc β1,2-Man α1,3
GlcNAcMan3GlcNAc2
GOLGI
β1,2 GnT II, β-1,4 GnT IV, β-1,4 GnT VI
GlcNAc β1,2
GlcNAc β1,4
Man α1,6
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
GlcNAc β1,4
Man α1,3
GlcNAc β1,2
Complex Glycoprotein
β1,4 GalT
Gal β1,4-GlcNAc β1,2
Gal β1,4-GlcNAc β1,4
Man α1,6
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
Gal β1,4-GlcNAc β1,4
Man α1,3
Gal β1,4-GlcNAc β1,2
Complex Glycoprotein
α2,3 ST, α2,6ST
NANA Gal β1,4-GlcNAc β1,2
NANA Gal β1,4-GlcNAc β1,4
Man α1,6
Man β1,4-GlcNAc β1,4-GlcNAcβ1-Asn
NANA Gal β1,4-GlcNAc β1,4
Man α1,3
NANA Gal β1,4-GlcNAc β1,2
Complex Glycoprotein

FIG. 1B

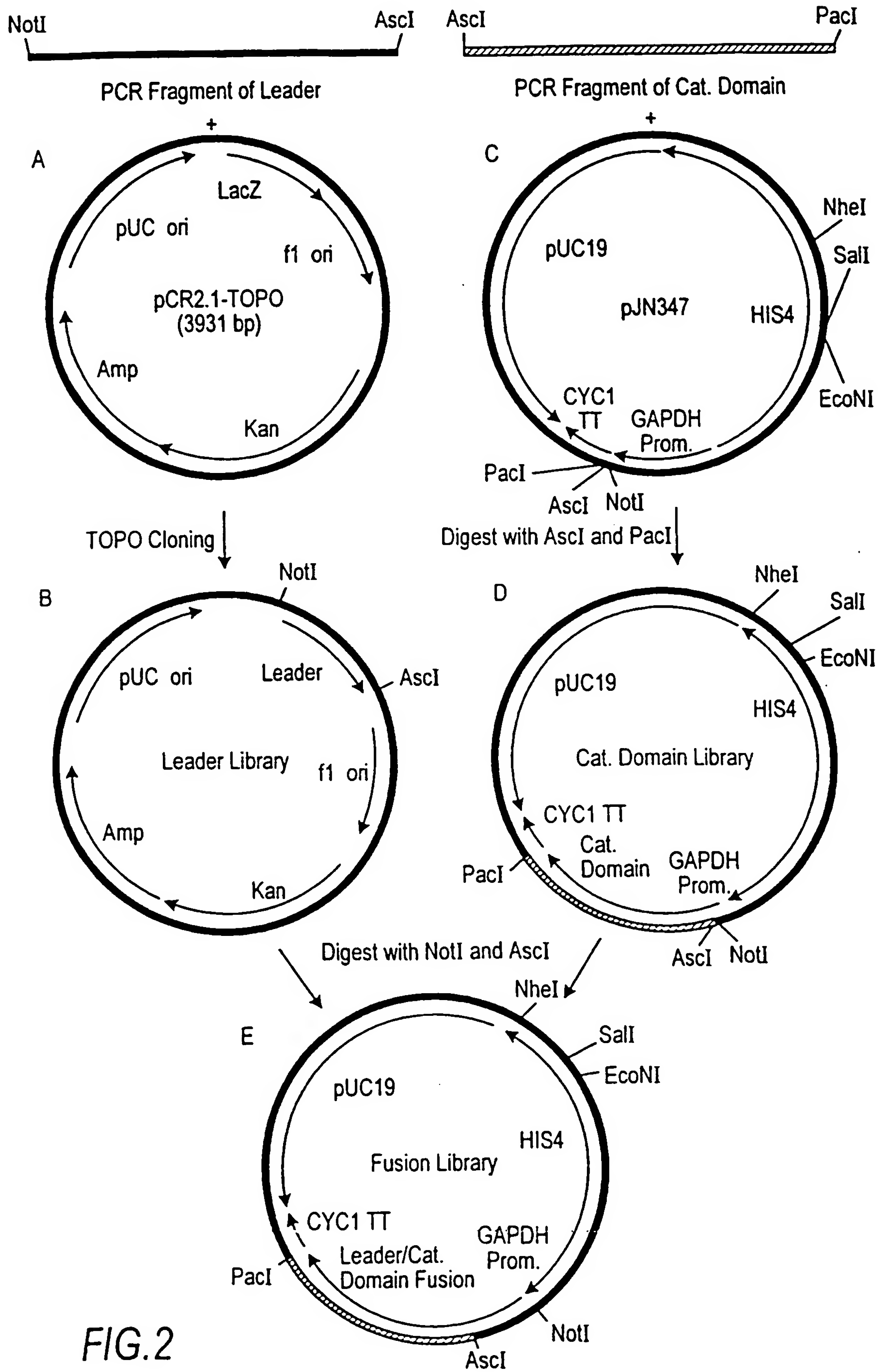

NotI
AscI
PCR Fragment of Leader
AscI
PacI
PCR Fragment of Cat. Domain
+
A
LacZ
pUC ori
f1 ori
pCR2.1-TOPO
(3931 bp)
Amp
Kan
+
C
NheI
SalI
pUC19
pJN347
HIS4
EcoNI
CYC1
TT
GAPDH
Prom.
PacI
AscI
NotI
TOPO Cloning
Digest with AscI and PacI
B
NotI
pUC ori
Leader
AscI
Leader Library
f1 ori
Amp
Kan
D
NheI
SalI
EcoNI
pUC19
HIS4
Cat. Domain Library
CYC1 TT
Cat.
Domain
GAPDH
Prom.
PacI
AscI
NotI
Digest with NotI and AscI
E
NheI
SalI
EcoNI
pUC19
HIS4
Fusion Library
CYC1 TT
GAPDH
Prom.
Leader/Cat.
Domain Fusion
PacI
NotI
AscI

FIG.2

M. musculus alpha-1,2-mannosidase IA open reading frame. The transmembrane and catalytic domains are highlighted in bold respectively. The sequence of the primers used to generate the N-terminal truncations are highlighted by underlining and the start of each respective protein fragment indicated by an arrow.

```
1    atgcccgtgggggcctgttgccgctcttcagtagccctggggcggcggcctgggcagtggcctgggcgggggcttggcggcgggaggaagggg
1  ▶ M  P  V  G  G  L  L  P  L  F  S  S  P  G  G  G  G  L  G  S  G  L  G  G  G  L  G  G  G  R  K  G
97   tctggccccgctgccttccgcctcaccgagaagttcgtgctgctgctggtgttcagcgccttcatcacgctctgcttcggggcaatc
33 ▶ S  G  P  A  A  F  R  L  T  E  K  F  V  L  L  L  V  F  S  A  F  I  T  L  C  F  G  A  I
184  ttcttcctgcctgactcctccaagctgctcagcggggtcctgttccactccaaccctgccttgcagccgccggcggagcacaagcccgggctcg
62 ▶ F  F  L  P  D  S  S  K  L  L  S  G  V  L  F  H  S  N  P  A  L  Q  P  P  A  E  H  K  P  G  L
     d65 primer
278  gggcgcgtgcggaggatgccgccgaggggagagtccggcaccgcgaggaaggcgcgcctggggaccctggagctggactggaagacaacttagcca
93 ▶ G  A  R  A  E  D  A  A  E  G  R  V  R  H  R  E  E  G  A  P  G  D  P  G  A  G  L  E  D  N  L  A
                 d105 primer
374  ggatccgcgaaaaccacgagcgggctctcagggaagccaaggagaccctgcagaagctgccggaggagatccaaagagacattctgctggagaagg
125▶ R  I  R  E  N  H  E  R  A  L  R  E  A  K  E  T  L  Q  K  L  P  E  E  I  Q  R  D  I  L  L  E  K
470  aaaaggtggcccaggaccagctgcgtgacaaggatctgtttaggggcttgcccaaggtggacttcctgcccccgtcggggtagagaaccgggagc
157▶ E  K  V  A  Q  D  Q  L  R  D  K  D  L  F  R  G  L  P  K  V  D  F  L  P  P  V  G  V  E  N  R  E
                                                                          d187 primer
566  ccgctgacgccaccatccgtgagaagagggcaaagatcaaagagatgatgacccatgcttggaataattataaacgctatgcgtggggc
189▶ P  A  D  A  T  I  R  E  K  R  A  K  I  K  E  M  M  T  H  A  W  N  N  Y  K  R  Y  A  W  G
```

FIG. 3

```
655  ttgaacgaactgaaacctatatcaaaagaaggccattcaagcagtttgtttggcaacatcaaaggagctacaatagtagatg
219▶ L  N  E  L  K  P  I  S  K  E  G  H  S  S  S  L  F  G  N  I  K  G  A  T  I  V  D
737  ccctggataccctttcattatgggcatgaagactgaatttcaagaagctaaatcgtggattaaaaatatttagatttaa
246▶A  L  D  T  L  F  I  M  G  M  K  T  E  F  Q  E  A  K  S  W  I  K  K  Y  L  D  F  N
819  tgtgaatgctgaagtttctgtttttgaagtcaacatacgcttcgtcggtggactgctgtcagcctactatttgtccggagag
273▶ V  N  A  E  V  S  V  F  E  V  N  I  R  F  V  G  G  L  L  S  A  Y  Y  L  S  G  E
901  gagatatttcgaaagaaagcagtggaacttgggtaaaattgctacctgcatttcatactccctctggaataccttgggcat
301▶ E  I  F  R  K  K  A  V  E  L  G  V  K  L  L  P  A  F  H  T  P  S  G  I  P  W  A
983  tgctgaatatgaaaagtgggatcgggcggaactggccctggcctctggaggcagcagtatcctggccgaatttggaactct
328▶L  L  N  M  K  S  G  I  G  R  N  W  P  W  A  S  G  G  S  S  I  L  A  E  F  G  T  L
1065 gcatttagagtttatgcacttgtcccacttatcaggagacccagtctttgccgaaaaggttatgaaaattcgaacagtgttg
355▶ H  L  E  F  M  H  L  S  H  L  S  G  D  P  V  F  A  E  K  V  M  K  I  R  T  V  L
1147 aacaaactggacaaaccagaaggcctttatcctaactatctgaaccccagtagtggacagtggggtcaacatcatgtgtcgg
383▶ N  K  L  D  K  P  E  G  L  Y  P  N  Y  L  N  P  S  S  G  Q  W  G  Q  H  H  V  S
1229 ttggaggacttggagacagctttttatgaatatttgcttaaggcgtggttaatgtctgacaagacagatctcgaagccaagaa
410▶V  G  G  L  G  D  S  F  Y  E  Y  L  L  K  A  W  L  M  S  D  K  T  D  L  E  A  K  K
1311 gatgtatttttgatgctgttcaggccatcgagactcacttgatccgcaagtcaagtggggggactaacgtacatcgcagagtgg
437▶ M  Y  F  D  A  V  Q  A  I  E  T  H  L  I  R  K  S  S  G  G  L  T  Y  I  A  E  W
1393 aagggggcctcctggaacacaagatgggccacctgacgtgctttgcaggaggcatgtttgcacttggggcagatggagctc
465▶ K  G  G  L  L  E  H  K  M  G  H  L  T  C  F  A  G  G  M  F  A  L  G  A  D  G  A
1475 cggaagcccgggcccaacactaccttgaactcggagctgaaattgcccgcacttgtcatgaatcttataatcgtacatatgt
492▶P  E  A  R  A  Q  H  Y  L  E  L  G  A  E  I  A  R  T  C  H  E  S  Y  N  R  T  Y  V
1557 gaagttgggaccggaagcgtttcgatttgatggcggtgtggaagctattgccacgaggcaaaatgaaaagtattacatctta
519▶ K  L  G  P  E  A  F  R  F  D  G  G  V  E  A  I  A  T  R  Q  N  E  K  Y  Y  I  L
1639 cggcccgaggtcatcgagacatacatgtacatgtggcgactgactcacgaccccaagtacaggacctgggcctgggaagccg
547▶ R  P  E  V  I  E  T  Y  M  Y  M  W  R  L  T  H  D  P  K  Y  R  T  W  A  W  E  A
1721 tggaggctctagaaagtcactgcagagtgaacggaggctactcaggcttacgggatgtttacattgcccgtgagagttatga
574▶V  E  A  L  E  S  H  C  R  V  N  G  G  Y  S  G  L  R  D  V  Y  I  A  R  E  S  Y  D
1803 cgatgtccagcaaagtttcttcctggcagagacactgaagtatttgtacttgatattttccgatgatgaccttcttccacta
601▶ D  V  Q  Q  S  F  F  L  A  E  T  L  K  Y  L  Y  L  I  F  S  D  D  D  L  L  P  L
1885 gaacactggatcttcaacaccgaggctcatcctttccctatactccgtgaacagaagaaggaaattgatggcaaagagaaatga
629▶ E  H  W  I  F  N  T  E  A  H  P  F  P  I  L  R  E  Q  K  K  E  I  D  G  K  E  K
```

FIG. 3 CON'T

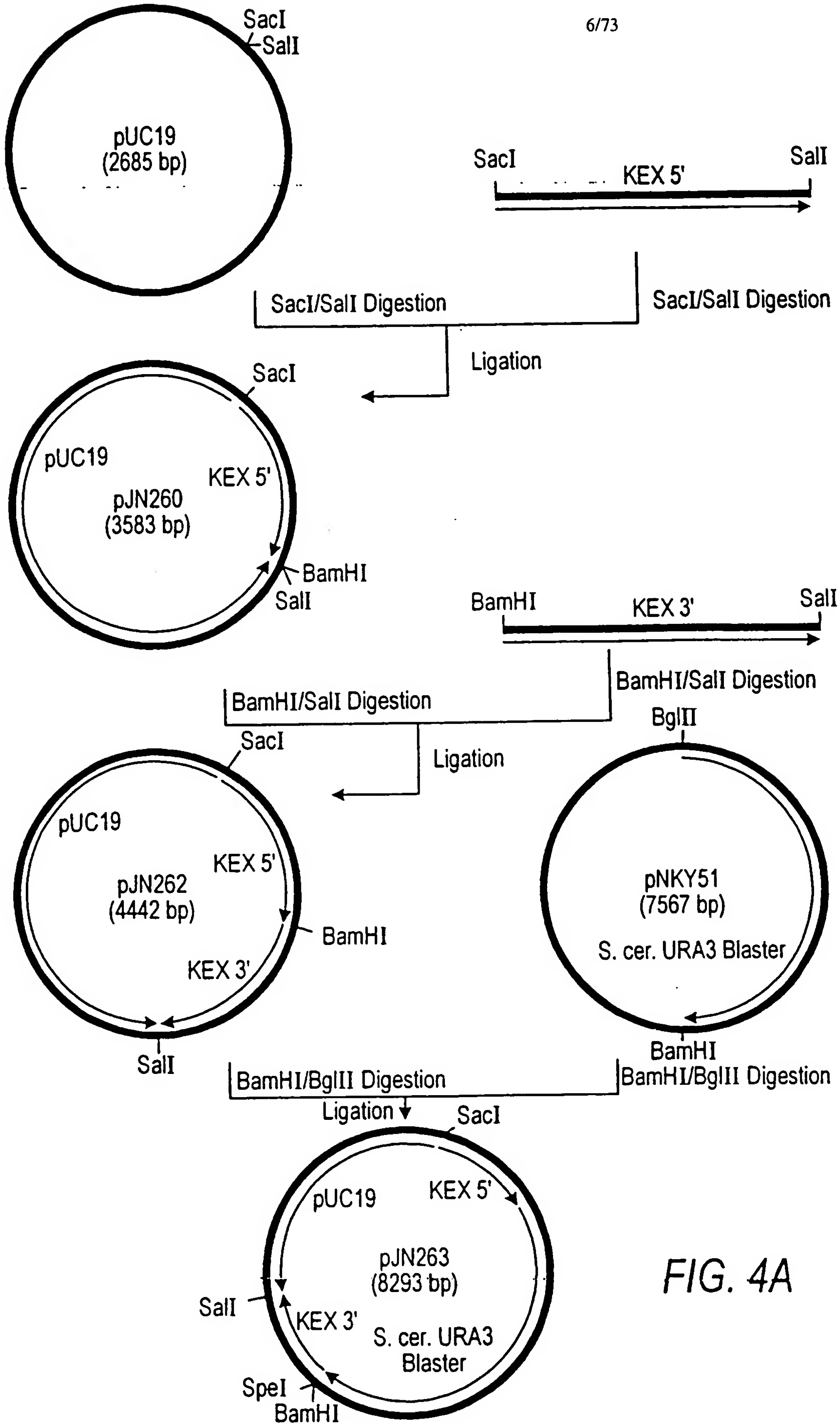

SacI
SalI
pUC19
(2685 bp)
SacI
KEX 5'
SalI
SacI/SalI Digestion
SacI/SalI Digestion
Ligation
SacI
pUC19
KEX 5'
pJN260
(3583 bp)
BamHI
SalI
BamHI
KEX 3'
SalI
BamHI/SalI Digestion
BamHI/SalI Digestion
BglII
SacI
Ligation
pUC19
KEX 5'
pJN262
(4442 bp)
BamHI
KEX 3'
SalI
pNKY51
(7567 bp)
S. cer. URA3 Blaster
BamHI
BamHI/BglII Digestion
BamHI/BglII Digestion
Ligation
SacI
pUC19
KEX 5'
pJN263
(8293 bp)
SalI
KEX 3'
S. cer. URA3
Blaster
SpeI
BamHI

FIG. 4A

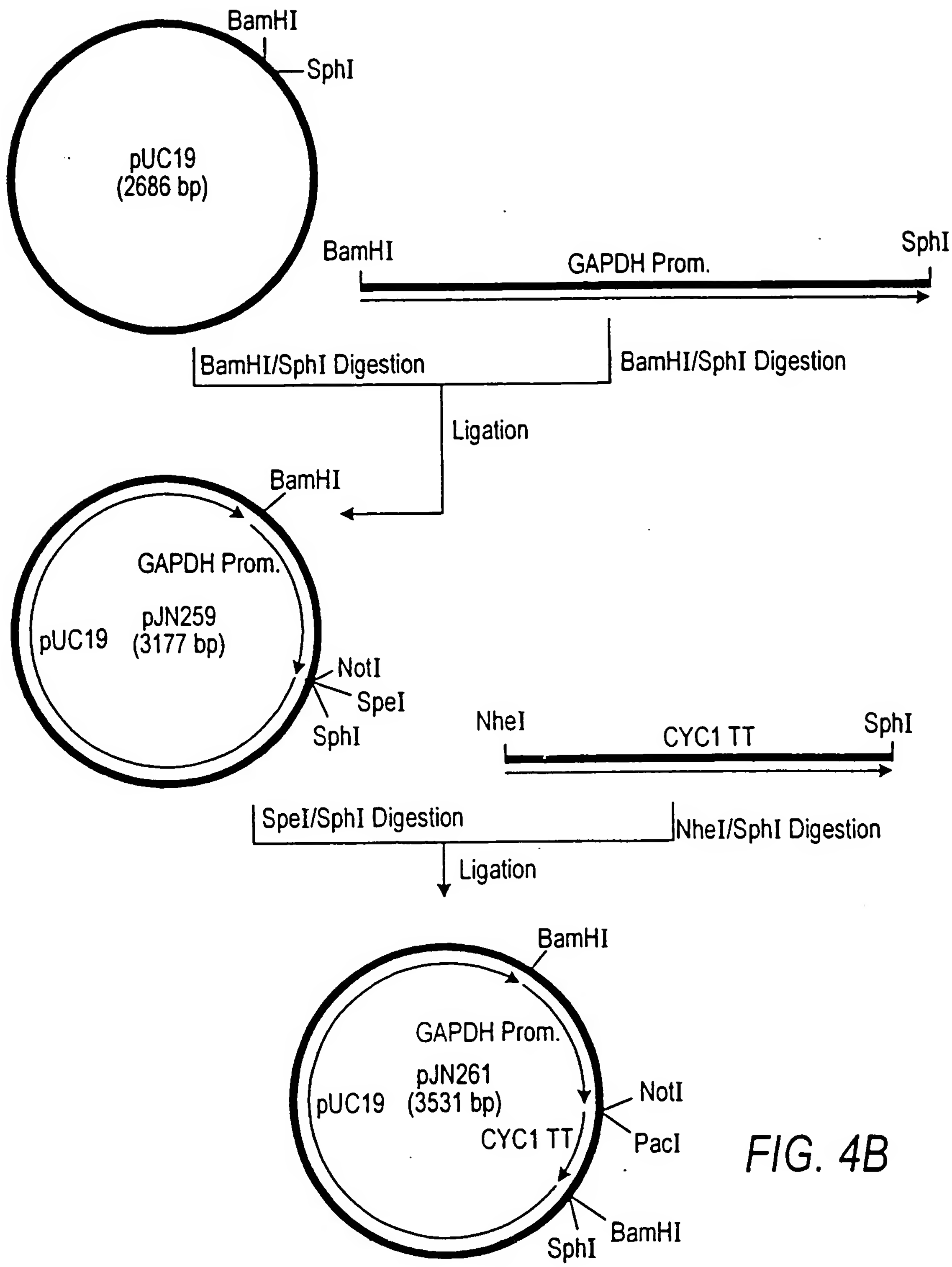

BamHI
SphI
pUC19
(2686 bp)
BamHI
GAPDH Prom.
SphI
BamHI/SphI Digestion
BamHI/SphI Digestion
Ligation
BamHI
GAPDH Prom.
pJN259
(3177 bp)
pUC19
NotI
SpeI
SphI
NheI
CYC1 TT
SphI
SpeI/SphI Digestion
NheI/SphI Digestion
Ligation
BamHI
GAPDH Prom.
pJN261
(3531 bp)
pUC19
CYC1 TT
NotI
PacI
SphI
BamHI

FIG. 4B

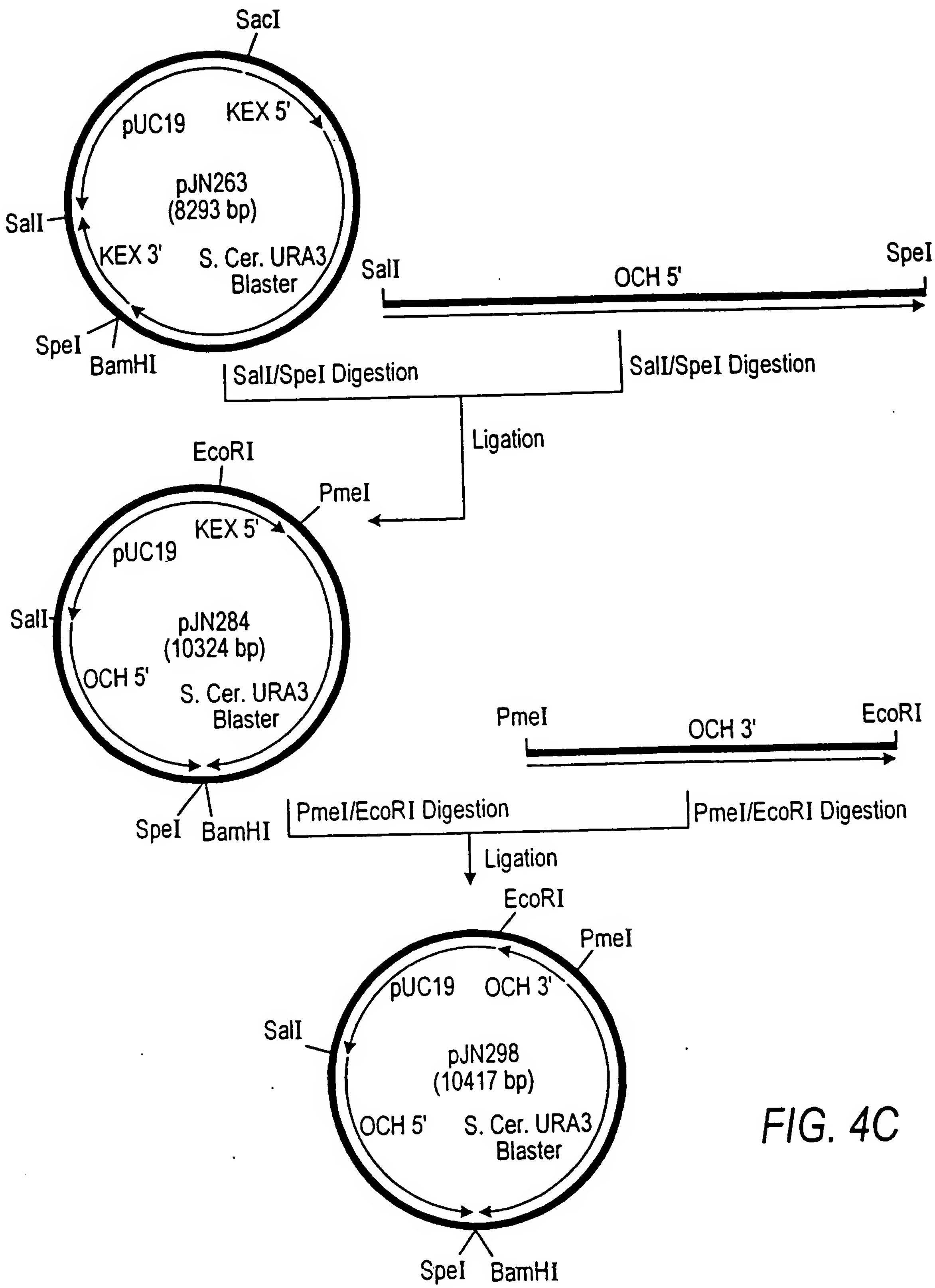

SacI
KEX 5'
pUC19
pJN263
(8293 bp)
SalI
KEX 3'
S. Cer. URA3
Blaster
SpeI
BamHI
SalI
OCH 5'
SpeI
SalI/SpeI Digestion
SalI/SpeI Digestion
Ligation
EcoRI
PmeI
KEX 5'
pUC19
SalI
pJN284
(10324 bp)
OCH 5'
S. Cer. URA3
Blaster
SpeI
BamHI
PmeI
OCH 3'
EcoRI
PmeI/EcoRI Digestion
PmeI/EcoRI Digestion
Ligation
EcoRI
PmeI
pUC19
OCH 3'
SalI
pJN298
(10417 bp)
OCH 5'
S. Cer. URA3
Blaster
SpeI
BamHI

FIG. 4C

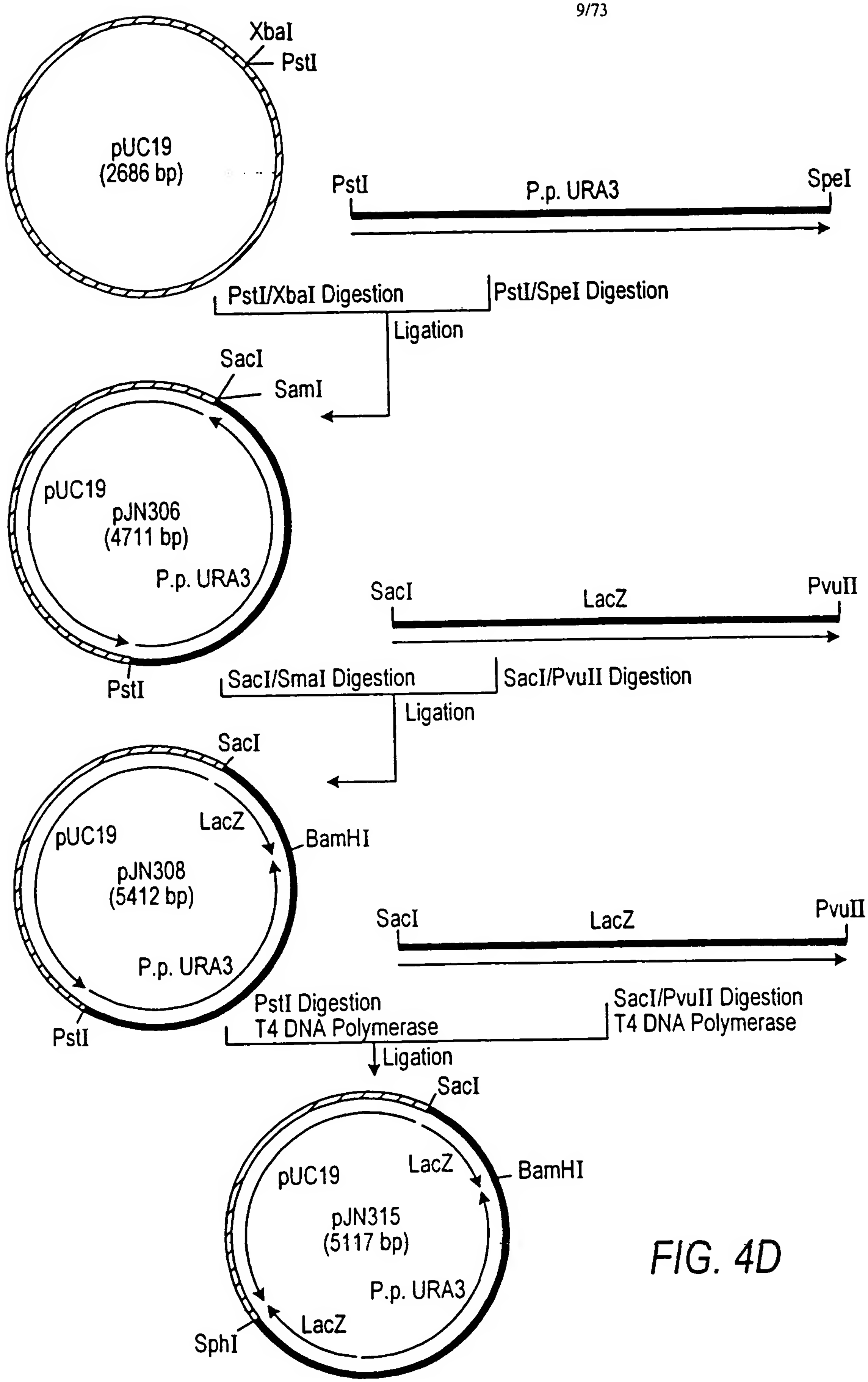

XbaI
PstI
pUC19
(2686 bp)
PstI
P.p. URA3
SpeI
PstI/XbaI Digestion
PstI/SpeI Digestion
Ligation
SacI
SamI
pUC19
pJN306
(4711 bp)
P.p. URA3
PstI
SacI
LacZ
PvuII
SacI/SmaI Digestion
SacI/PvuII Digestion
Ligation
SacI
pUC19
LacZ
BamHI
pJN308
(5412 bp)
P.p. URA3
PstI
SacI
LacZ
PvuII
PstI Digestion
T4 DNA Polymerase
SacI/PvuII Digestion
T4 DNA Polymerase
Ligation
SacI
pUC19
LacZ
BamHI
pJN315
(5117 bp)
P.p. URA3
LacZ
SphI

FIG. 4D

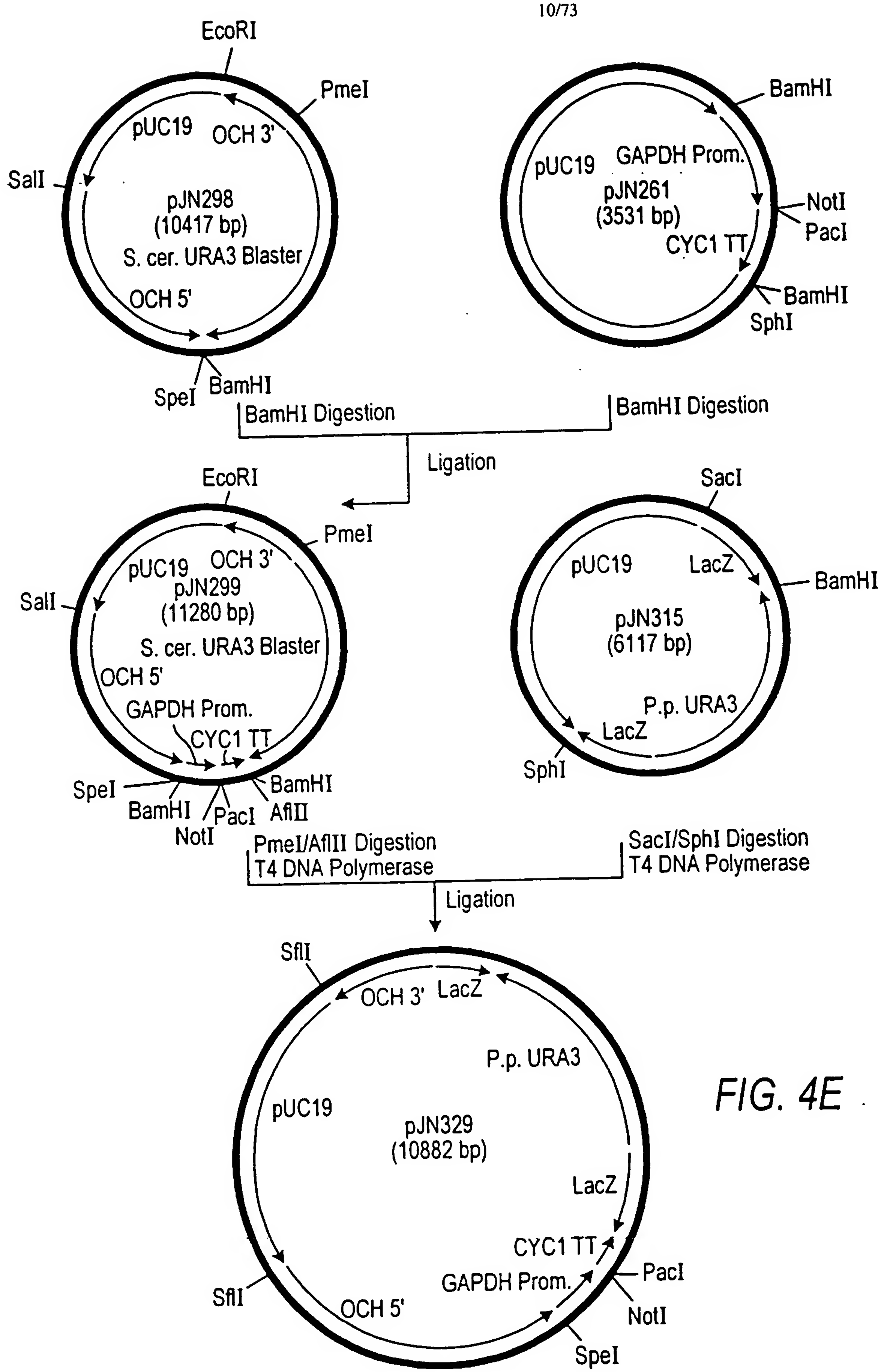

EcoRI
PmeI
pUC19
OCH 3'
SalI
pJN298
(10417 bp)
S. cer. URA3 Blaster
OCH 5'
SpeI
BamHI
BamHI
pUC19
GAPDH Prom.
pJN261
(3531 bp)
NotI
PacI
CYC1 TT
BamHI
SphI
BamHI Digestion
BamHI Digestion
Ligation
EcoRI
PmeI
pUC19
OCH 3'
pJN299
(11280 bp)
SalI
S. cer. URA3 Blaster
OCH 5'
GAPDH Prom.
CYC1 TT
SpeI
BamHI
BamHI
NotI
PacI
AflII
SacI
pUC19
LacZ
BamHI
pJN315
(6117 bp)
P.p. URA3
LacZ
SphI
PmeI/AflII Digestion
T4 DNA Polymerase
SacI/SphI Digestion
T4 DNA Polymerase
Ligation
SfII
OCH 3'
LacZ
P.p. URA3
pUC19
pJN329
(10882 bp)
LacZ
CYC1 TT
PacI
GAPDH Prom.
SfII
OCH 5'
NotI
SpeI

FIG. 4E

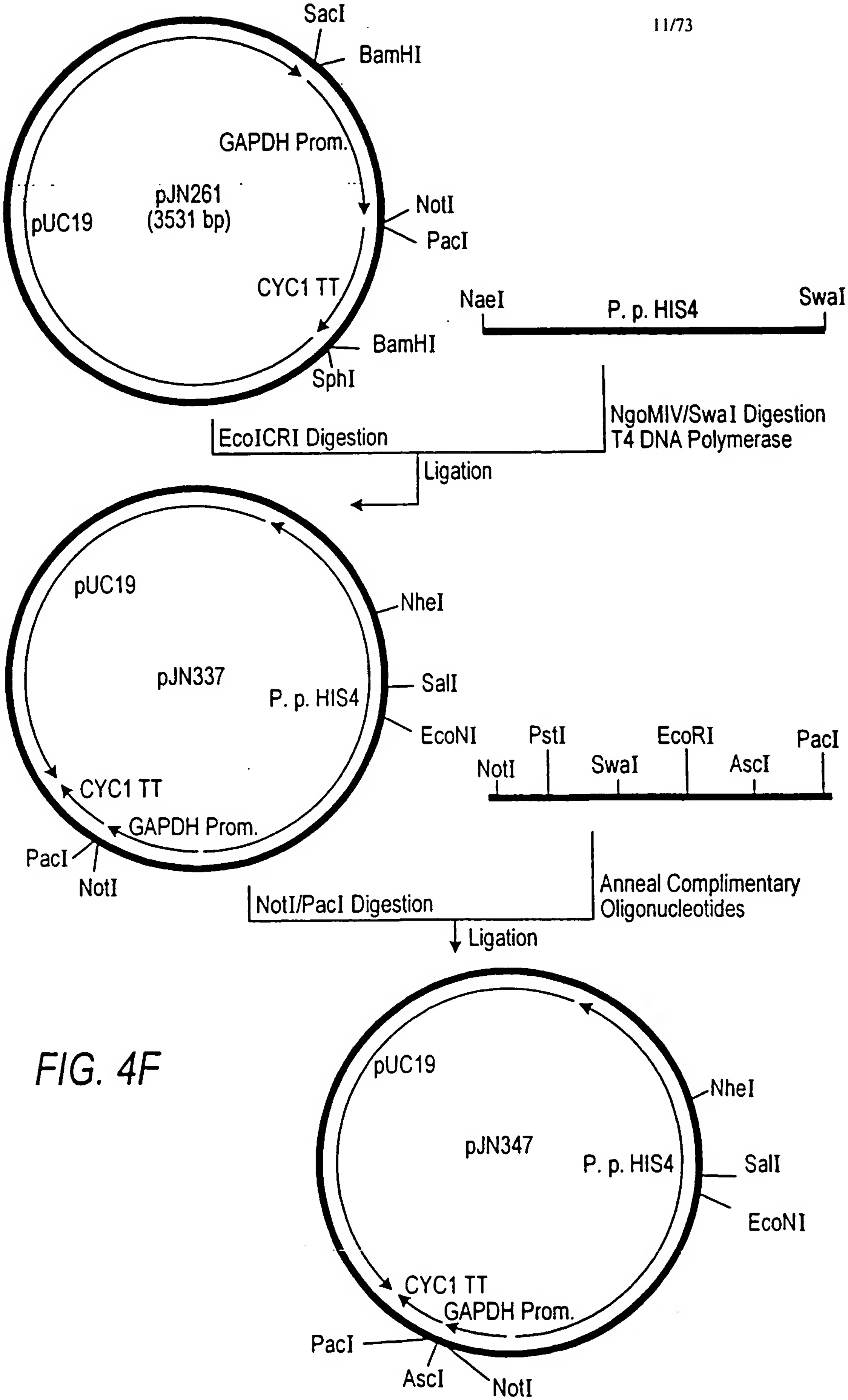

SacI
BamHI
GAPDH Prom.
pJN261
(3531 bp)
pUC19
NotI
PacI
CYC1 TT
BamHI
SphI
NaeI
P. p. HIS4
SwaI
NgoMIV/SwaI Digestion
T4 DNA Polymerase
EcoICRI Digestion
Ligation
pUC19
NheI
pJN337
P. p. HIS4
SalI
EcoNI
CYC1 TT
GAPDH Prom.
PacI
NotI
NotI
PstI
SwaI
EcoRI
AscI
PacI
Anneal Complimentary
Oligonucleotides
NotI/PacI Digestion
Ligation
pUC19
NheI
pJN347
P. p. HIS4
SalI
EcoNI
CYC1 TT
GAPDH Prom.
PacI
AscI
NotI

FIG. 4F

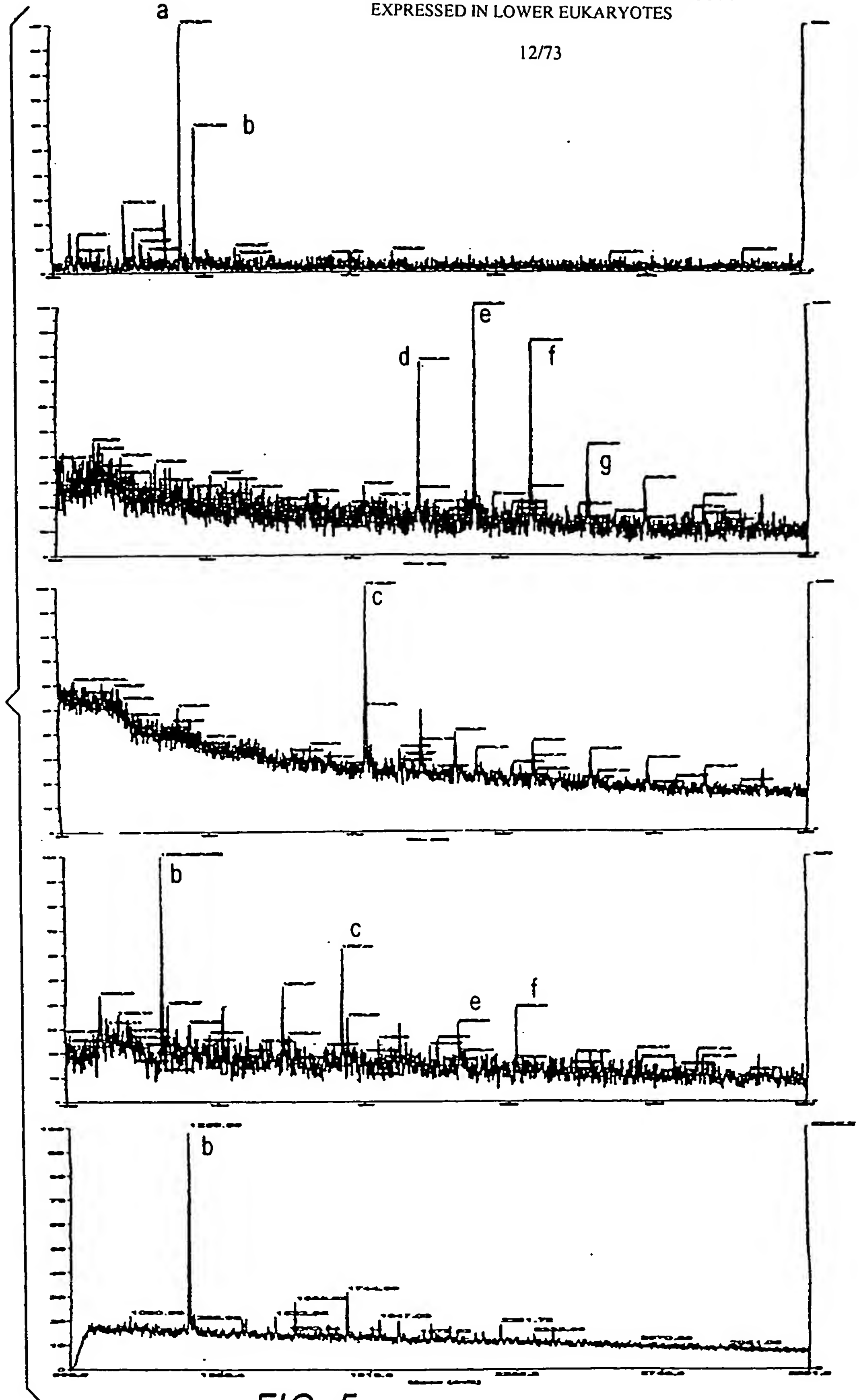


FIG. 5

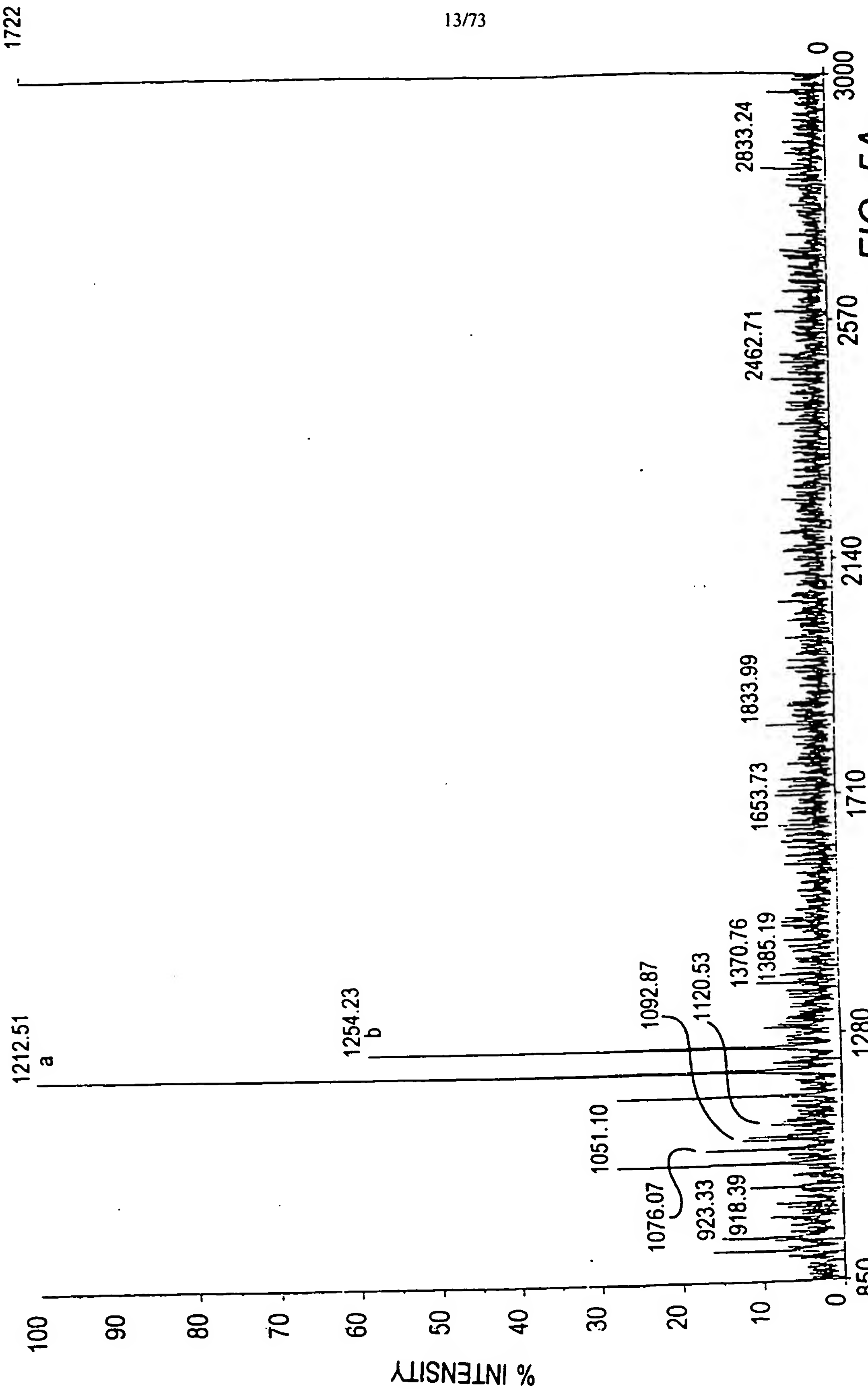

1722
% INTENSITY
MASS (m/z)
0
10
20
30
40
50
60
70
80
90
100
850
1280
1710
2140
2570
3000
1212.51
a
1254.23
b
1092.87
1120.53
1370.76
1385.19
1051.10
1076.07
923.33
918.39
1653.73
1833.99
2462.71
2833.24

FIG. 5A

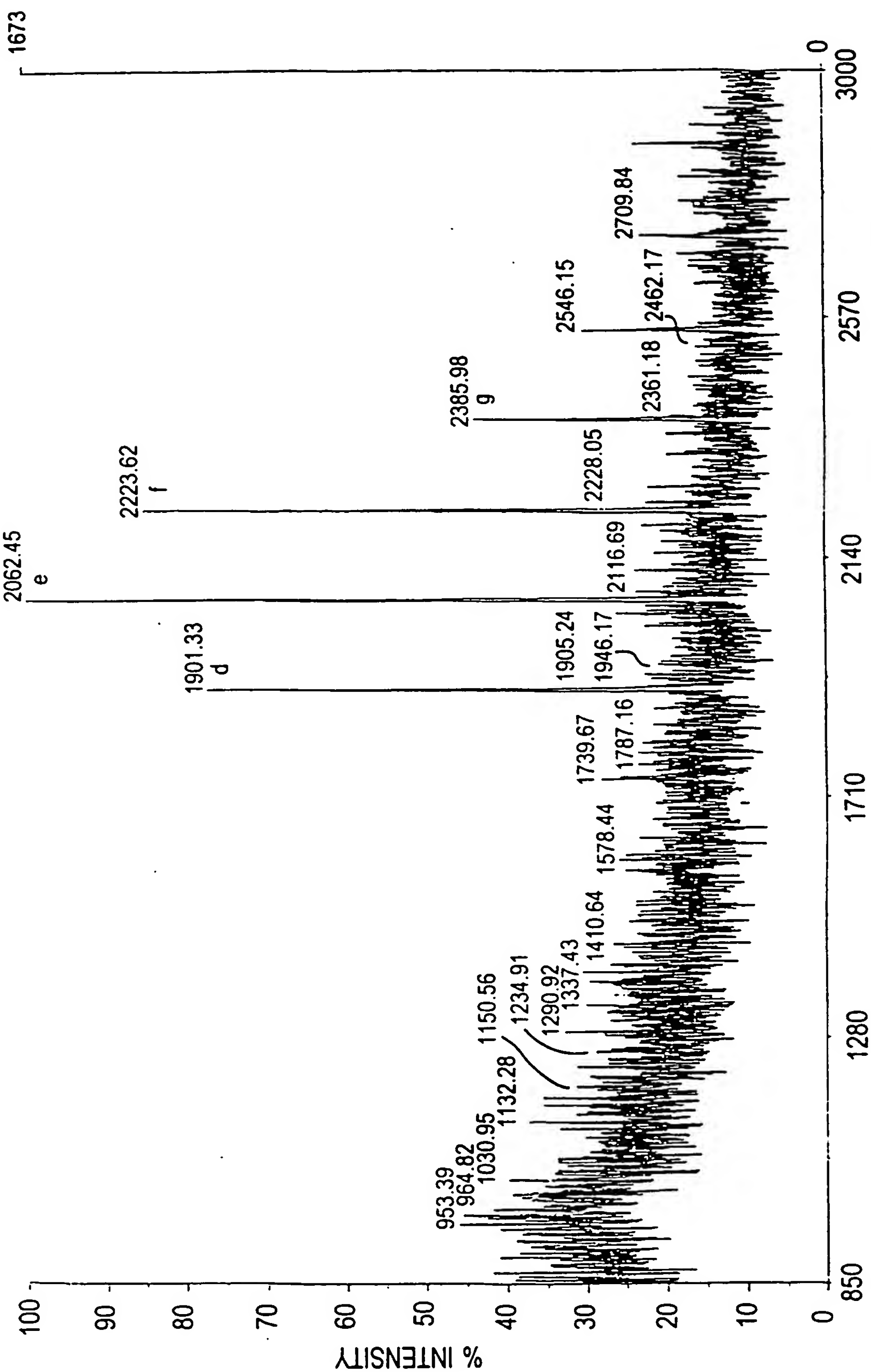

1673
2062.45
e
2223.62
f
2385.98
g
1901.33
d
2546.15
2709.84
2462.17
2361.18
2228.05
2116.69
1905.24
1946.17
1739.67
1787.16
1578.44
1410.64
1337.43
1290.92
1234.91
1150.56
1132.28
1030.95
964.82
953.39
% INTENSITY
100
90
80
70
60
50
40
30
20
10
0
850
1280
1710
2140
2570
3000
MASS (m/z)

FIG. 5B

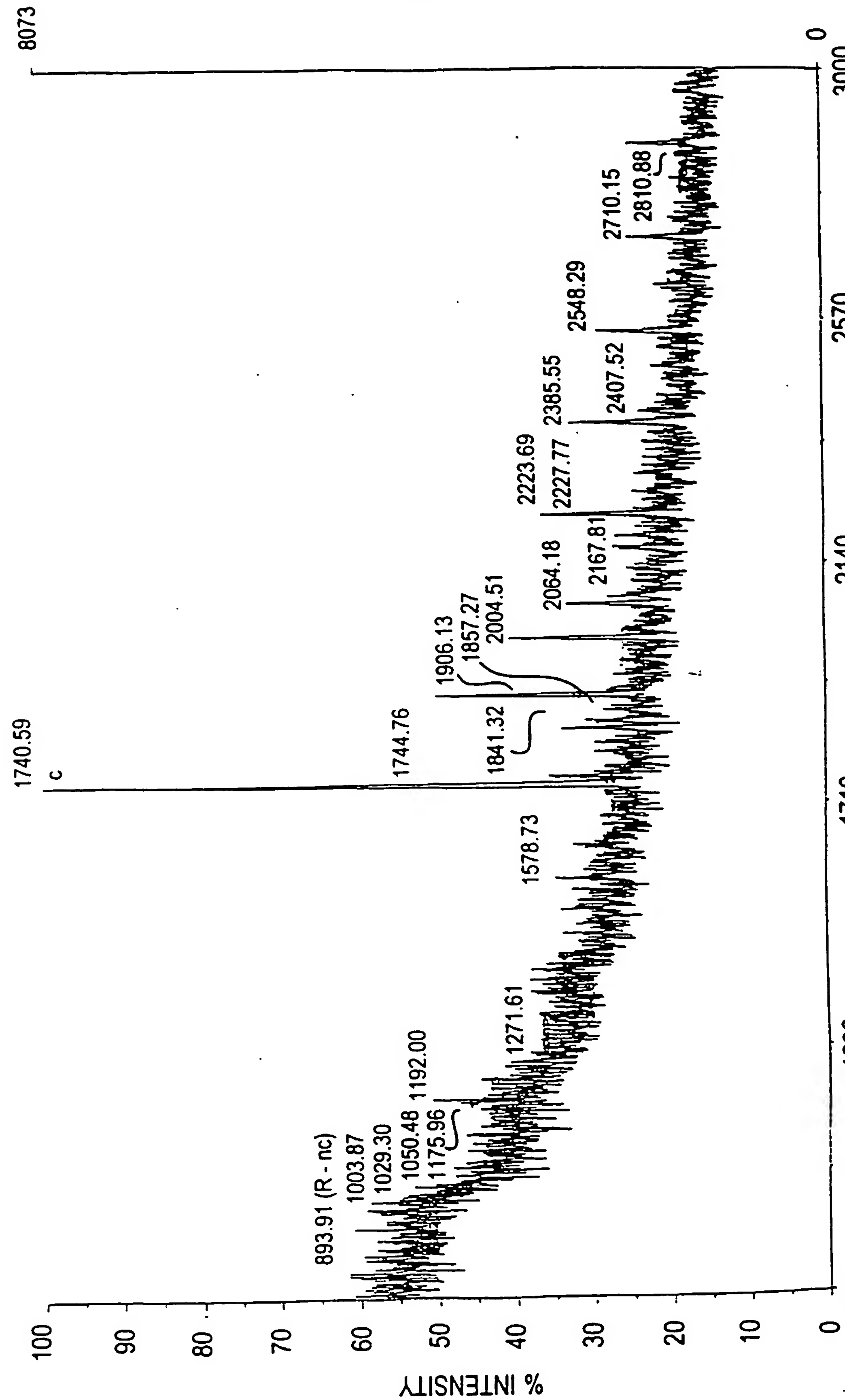

8073
0
1740.59
c
1744.76
1841.32
1906.13
1857.27
2004.51
2064.18
2167.81
2223.69
2227.77
2385.55
2407.52
2548.29
2710.15
2810.88
1578.73
1271.61
1192.00
1175.96
1050.48
1029.30
1003.87
893.91 (R - nc)
% INTENSITY
100
90
80
70
60
50
40
30
20
10
0
MASS (m/z)
850
1280
1710
2140
2570
3000

FIG. 5C

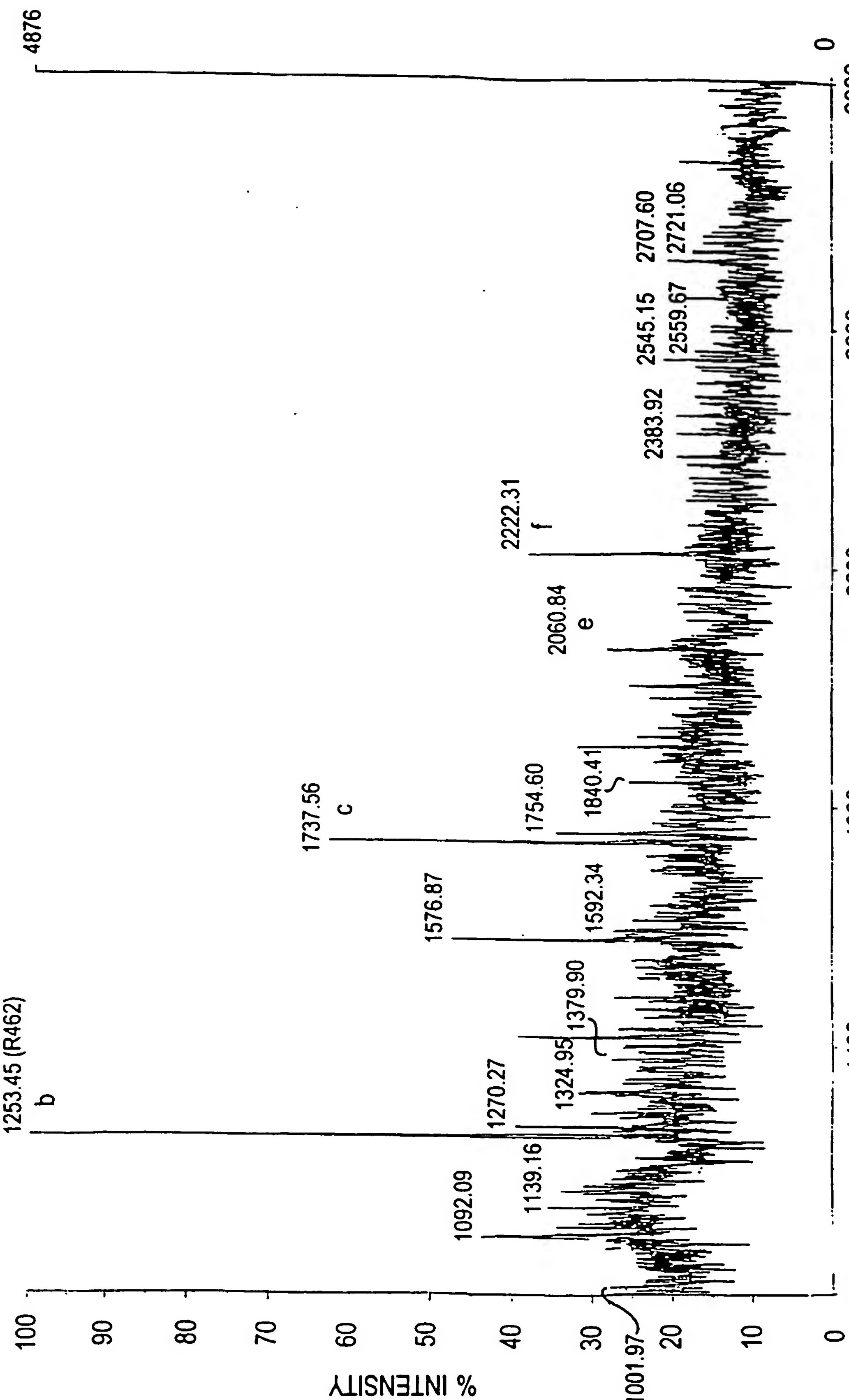

% INTENSITY
MASS (m/z)
0
10
20
30
40
50
60
70
80
90
100
1000
1400
1800
2200
2600
3000
4876
1001.97
1092.09
1139.16
1253.45 (R462)
b
1270.27
1324.95
1379.90
1576.87
1592.34
1737.56
c
1754.60
1840.41
2060.84
e
2222.31
f
2383.92
2545.15
2559.67
2707.60
2721.06

FIG. 5D

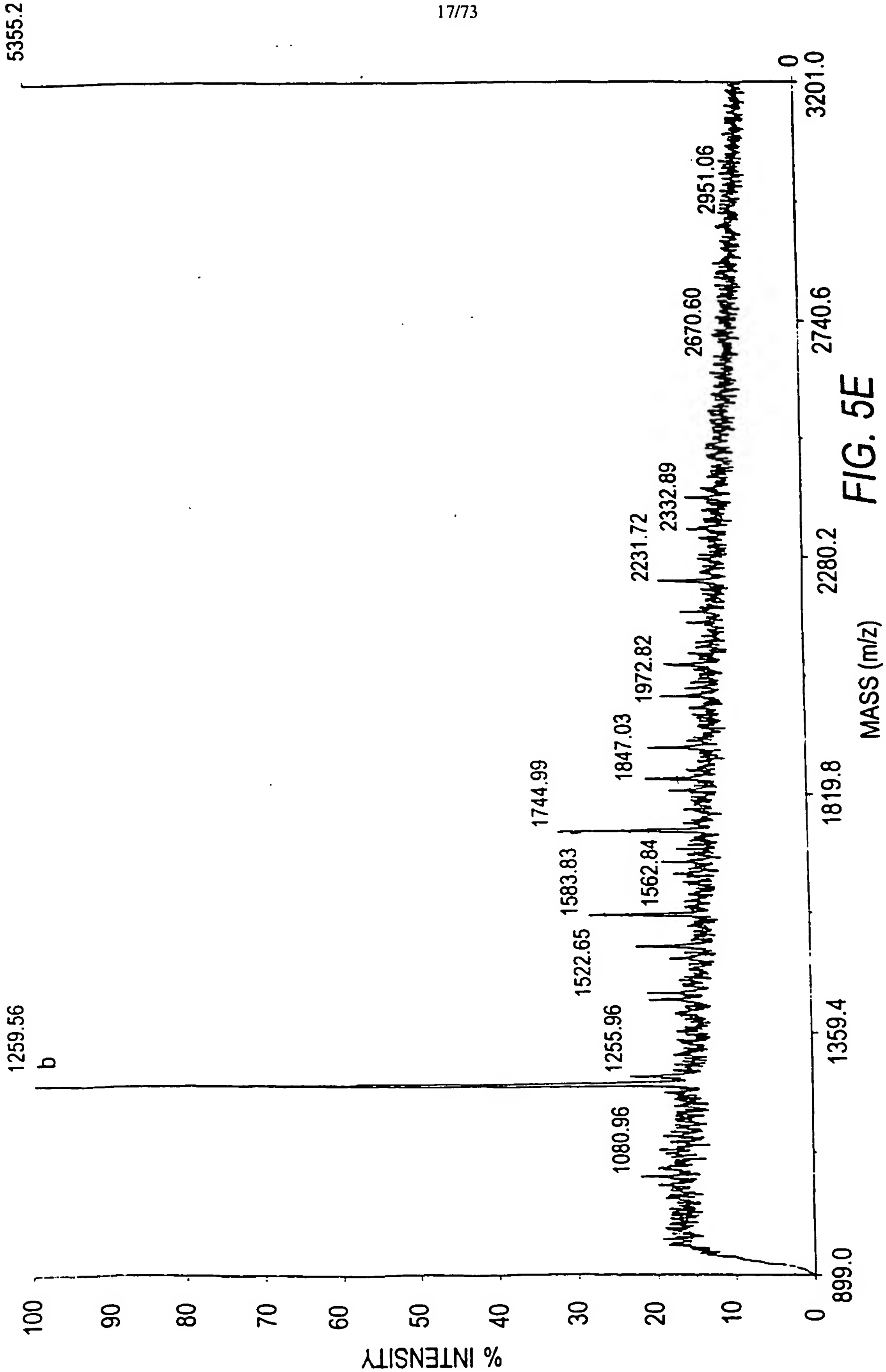

5355.2
% INTENSITY
100
90
80
70
60
50
40
30
20
10
0
MASS (m/z)
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
1259.56
b
1080.96
1255.96
1522.65
1583.83
1562.84
1744.99
1847.03
1972.82
2231.72
2332.89
2670.60
2951.06

FIG. 5E

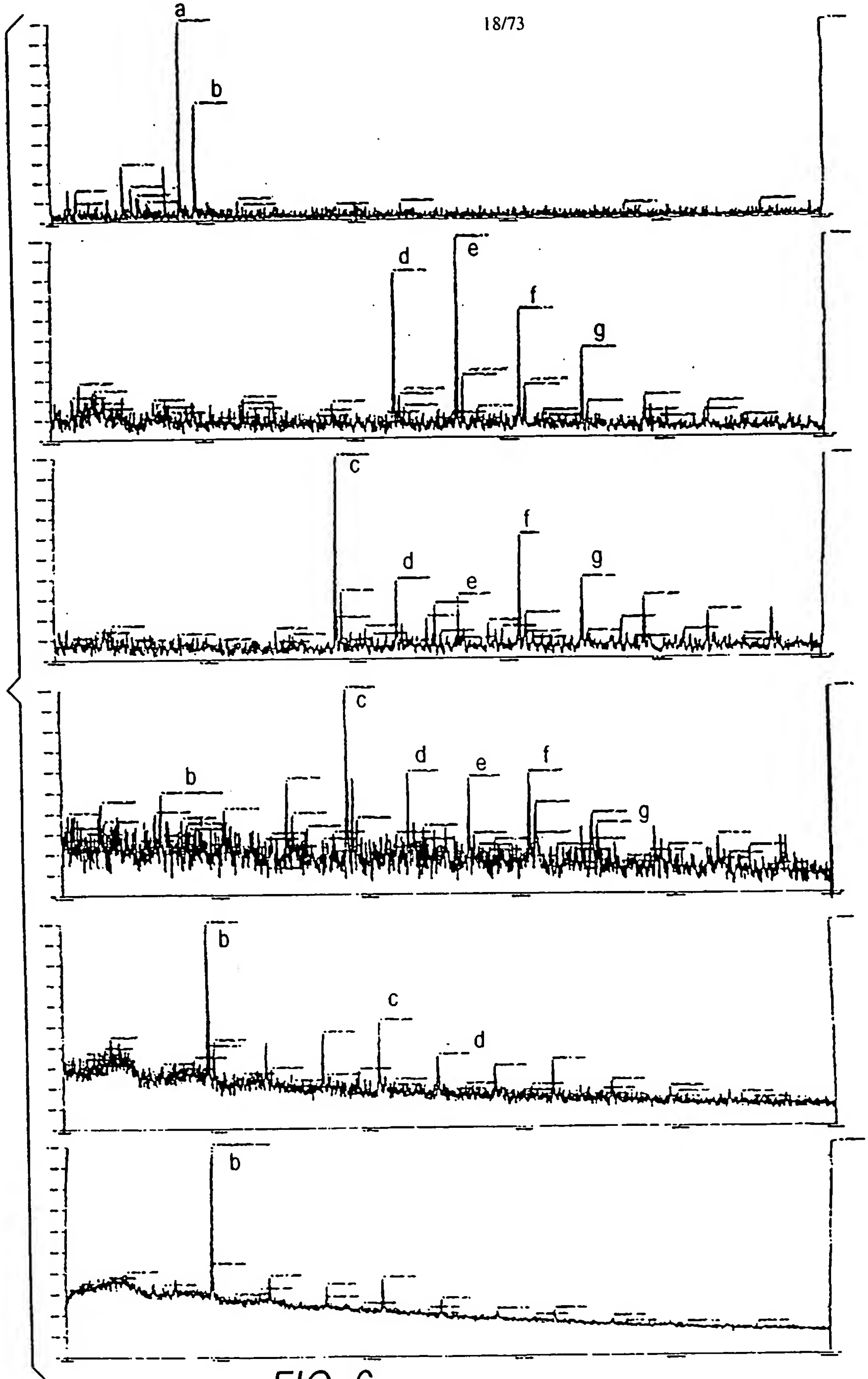
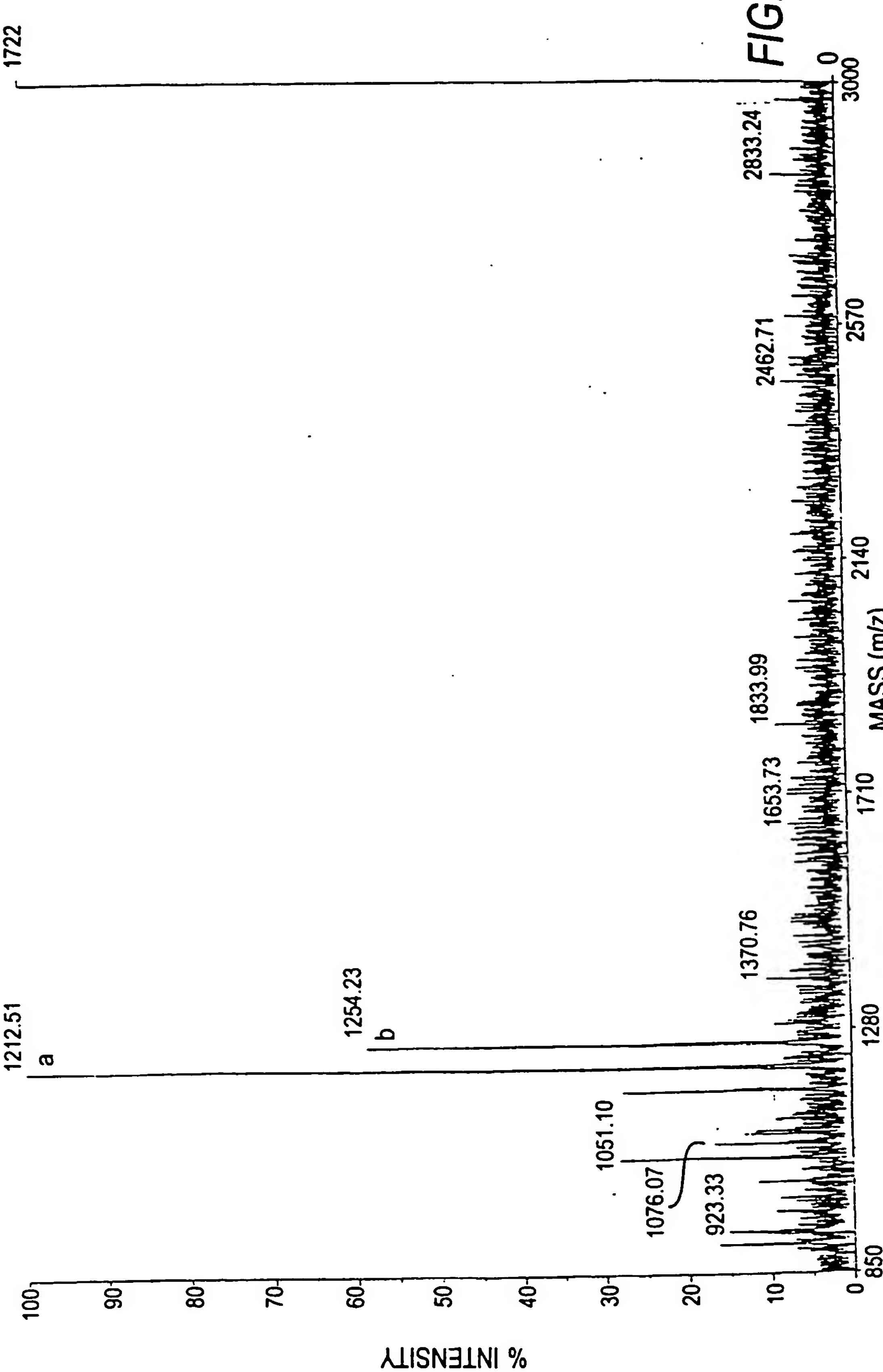


FIG. 6

FIG. 6A



1722
1212.51
a
1254.23
b
1370.76
1653.73
1833.99
2462.71
2833.24
1051.10
1076.07
923.33
% INTENSITY
MASS (m/z)
0
10
20
30
40
50
60
70
80
90
100
850
1280
1710
2140
2570
3000

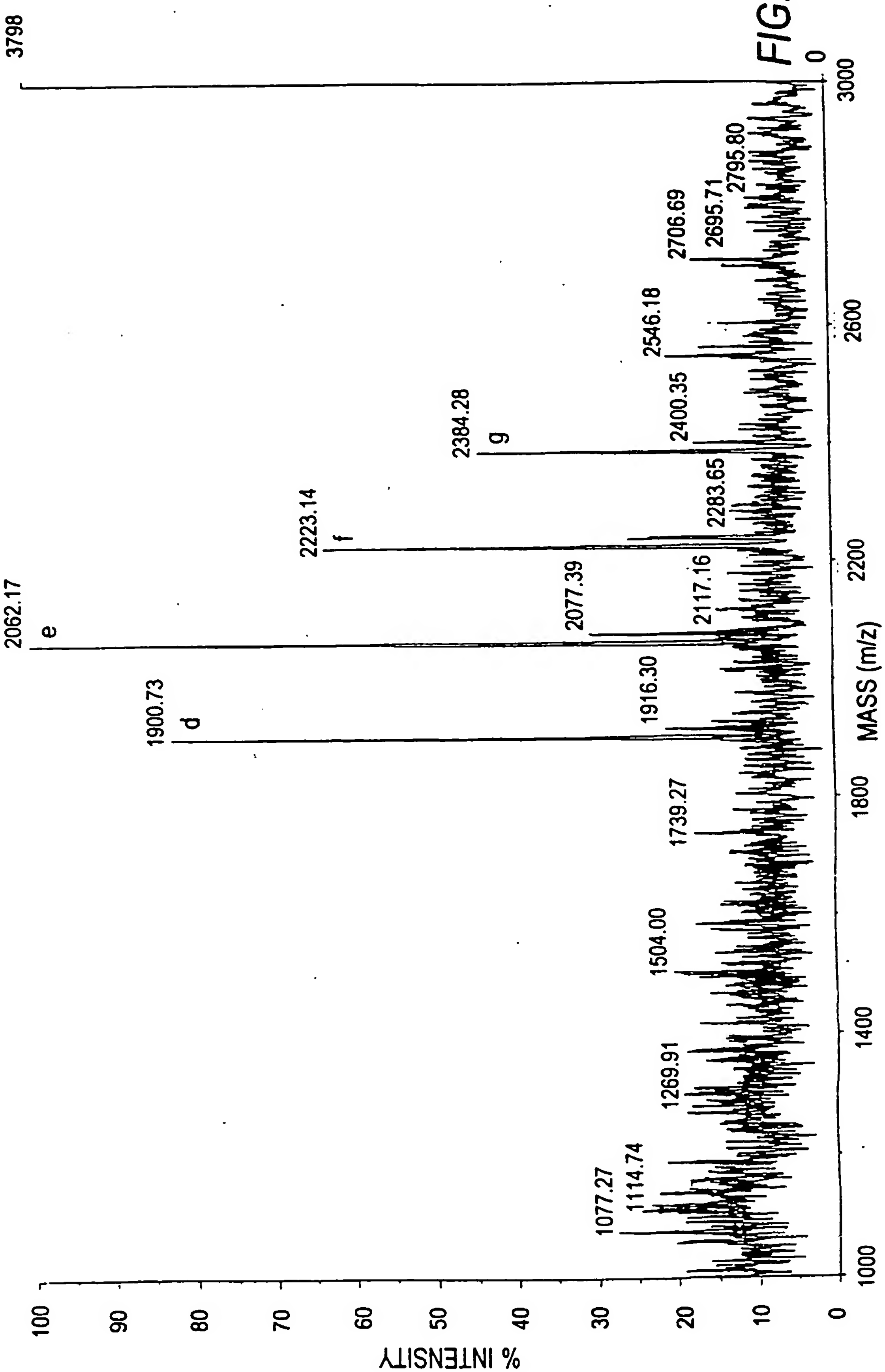
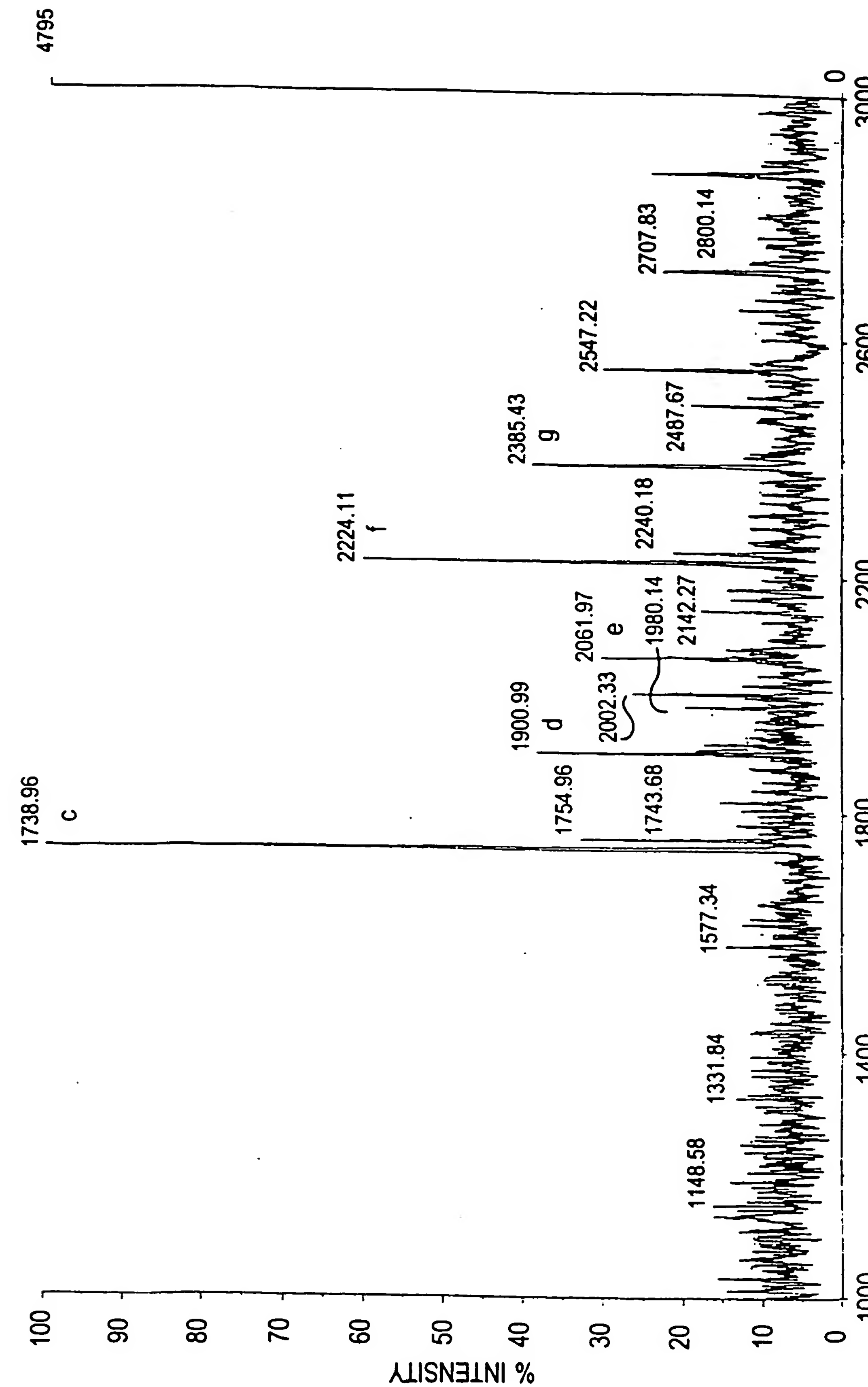

3798
2062.17
e
1900.73
d
2223.14
f
2384.28
g
2077.39
2117.16
1916.30
2283.65
2400.35
2546.18
2706.69
2695.71
2795.80
1739.27
1504.00
1269.91
1077.27
1114.74
% INTENSITY
100
90
80
70
60
50
40
30
20
10
0
MASS (m/z)
1000
1400
1800
2200
2600
3000
0

FIG. 6B



4795
1738.96
c
1754.96
1743.68
1900.99
d
2002.33
2061.97
e
1980.14
2142.27
2224.11
f
2240.18
2385.43
g
2487.67
2547.22
2707.83
2800.14
1577.34
1331.84
1148.58
% INTENSITY
MASS (m/z)

FIG. 6C

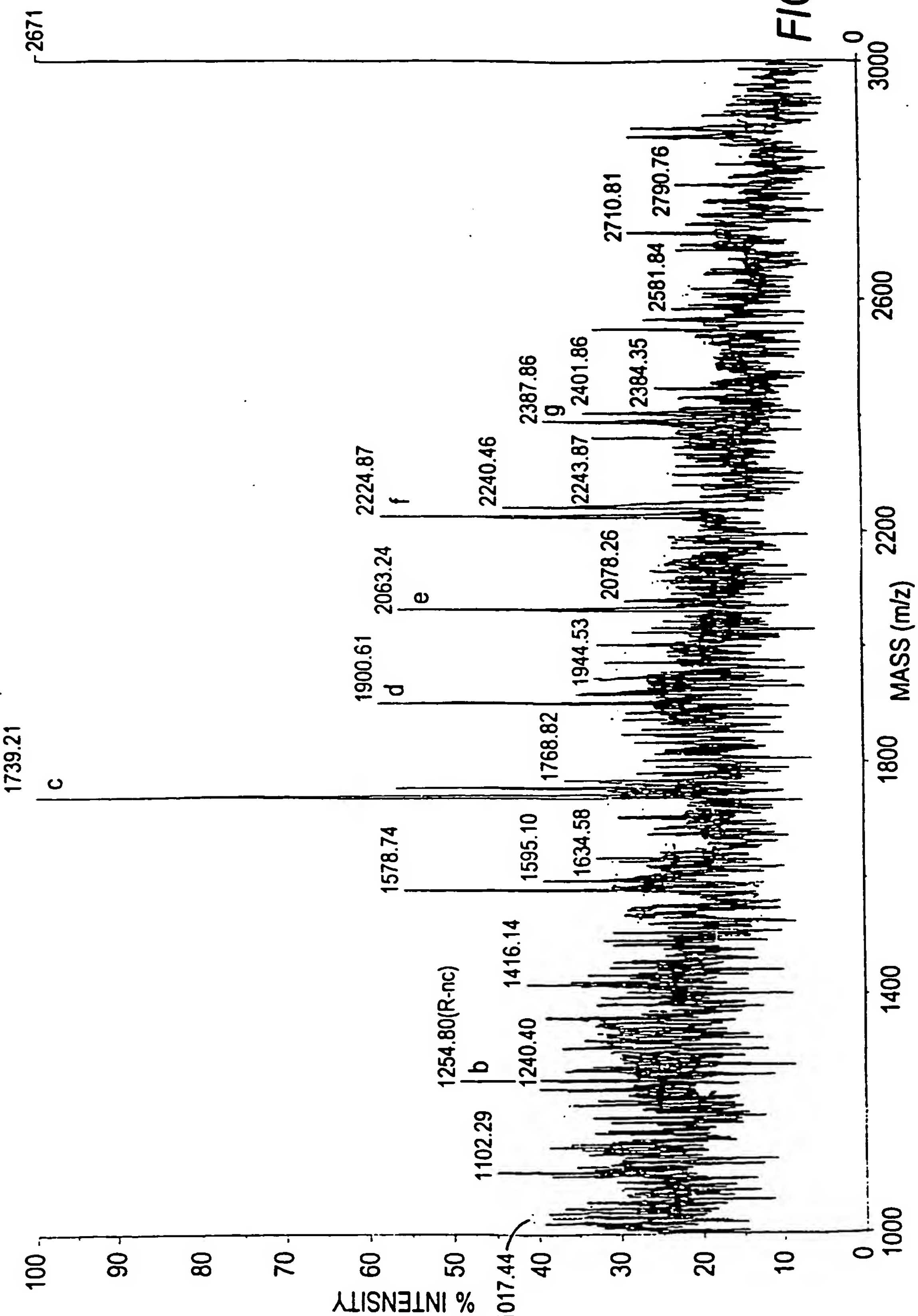
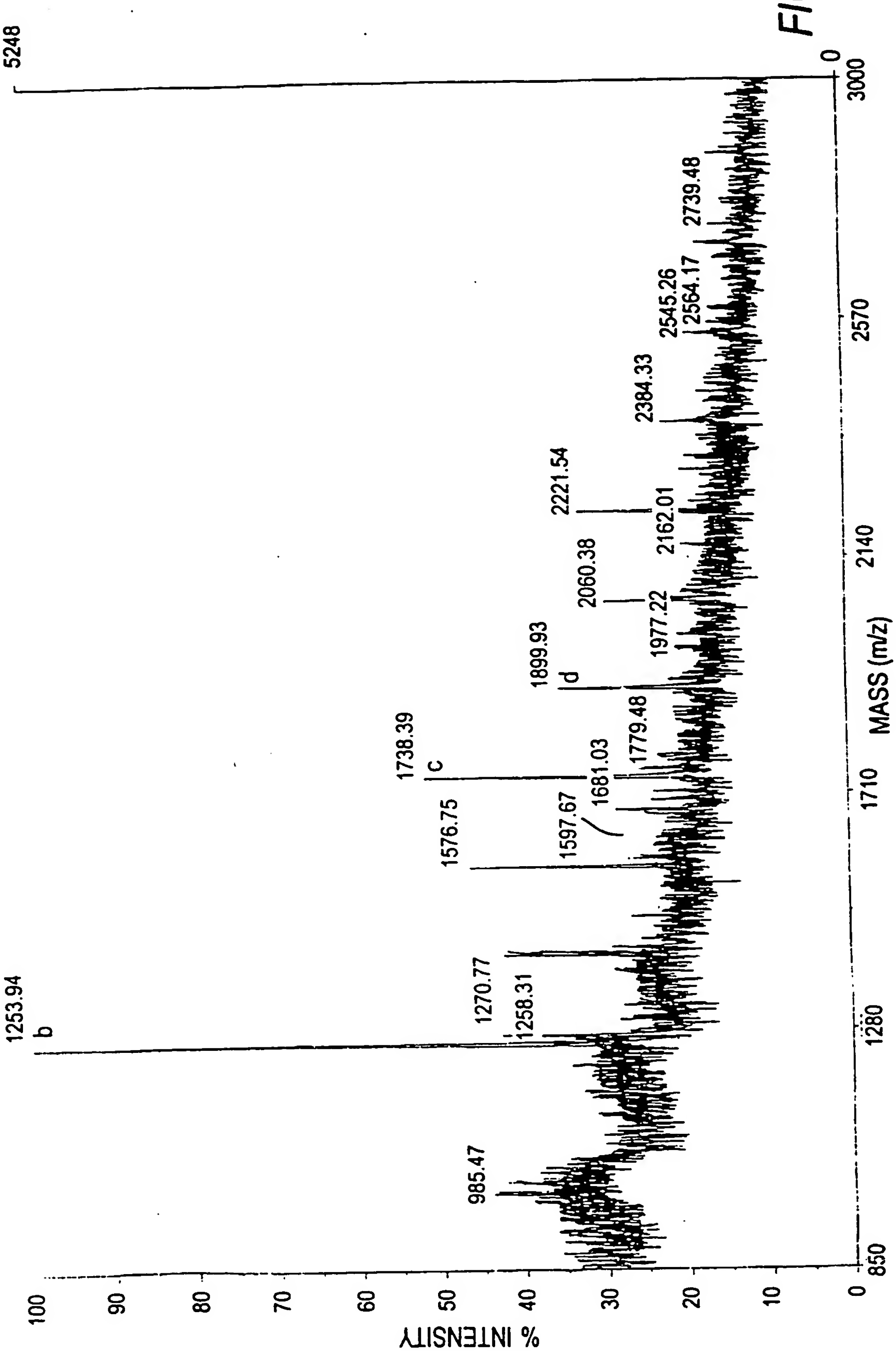
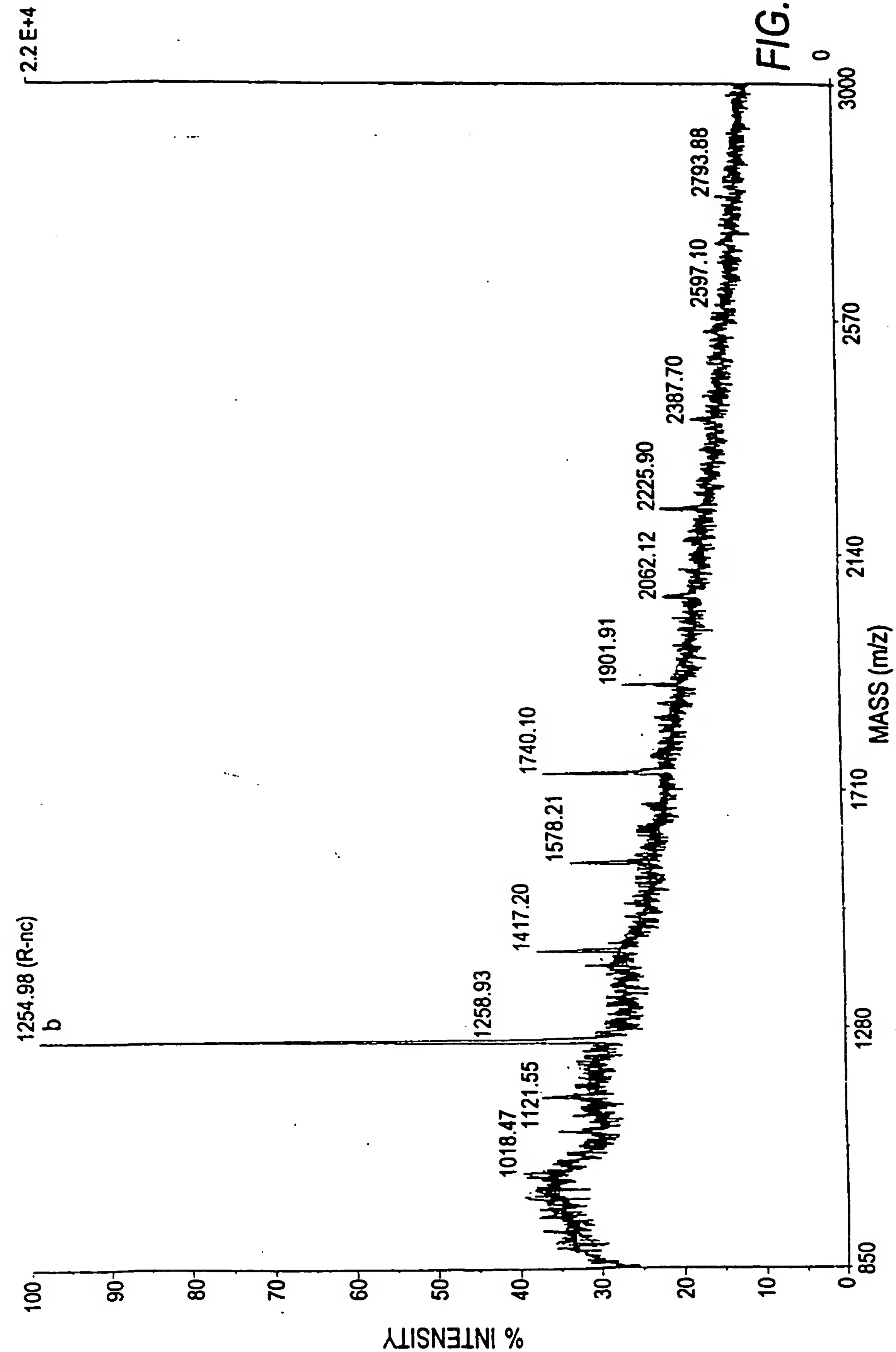

% INTENSITY
MASS (m/z)
100
90
80
70
60
50
40
30
20
10
0
1000
1400
1800
2200
2600
3000
1017.44
1102.29
1254.80(R-nc)
b
1240.40
1416.14
1578.74
1595.10
1634.58
1739.21
c
1768.82
1900.61
d
1944.53
2063.24
e
2078.26
2224.87
f
2240.46
2243.87
2387.86
g
2401.86
2384.35
2581.84
2710.81
2790.76
2671

FIG. 6D

FIG. 6E


5248
985.47
1253.94
b
1258.31
1270.77
1576.75
1597.67
1681.03
1738.39
c
1779.48
1899.93
d
1977.22
2060.38
2162.01
2221.54
2384.33
2545.26
2564.17
2739.48
% INTENSITY
0
10
20
30
40
50
60
70
80
90
100
MASS (m/z)
850
1280
1710
2140
2570
3000
0



2.2 E+4
1254.98 (R-nc)
b
1258.93
1018.47
1121.55
1417.20
1578.21
1740.10
1901.91
2062.12
2225.90
2387.70
2597.10
2793.88
% INTENSITY
MASS (m/z)
850
1280
1710
2140
2570
3000
0
10
20
30
40
50
60
70
80
90
100

FIG. 6F

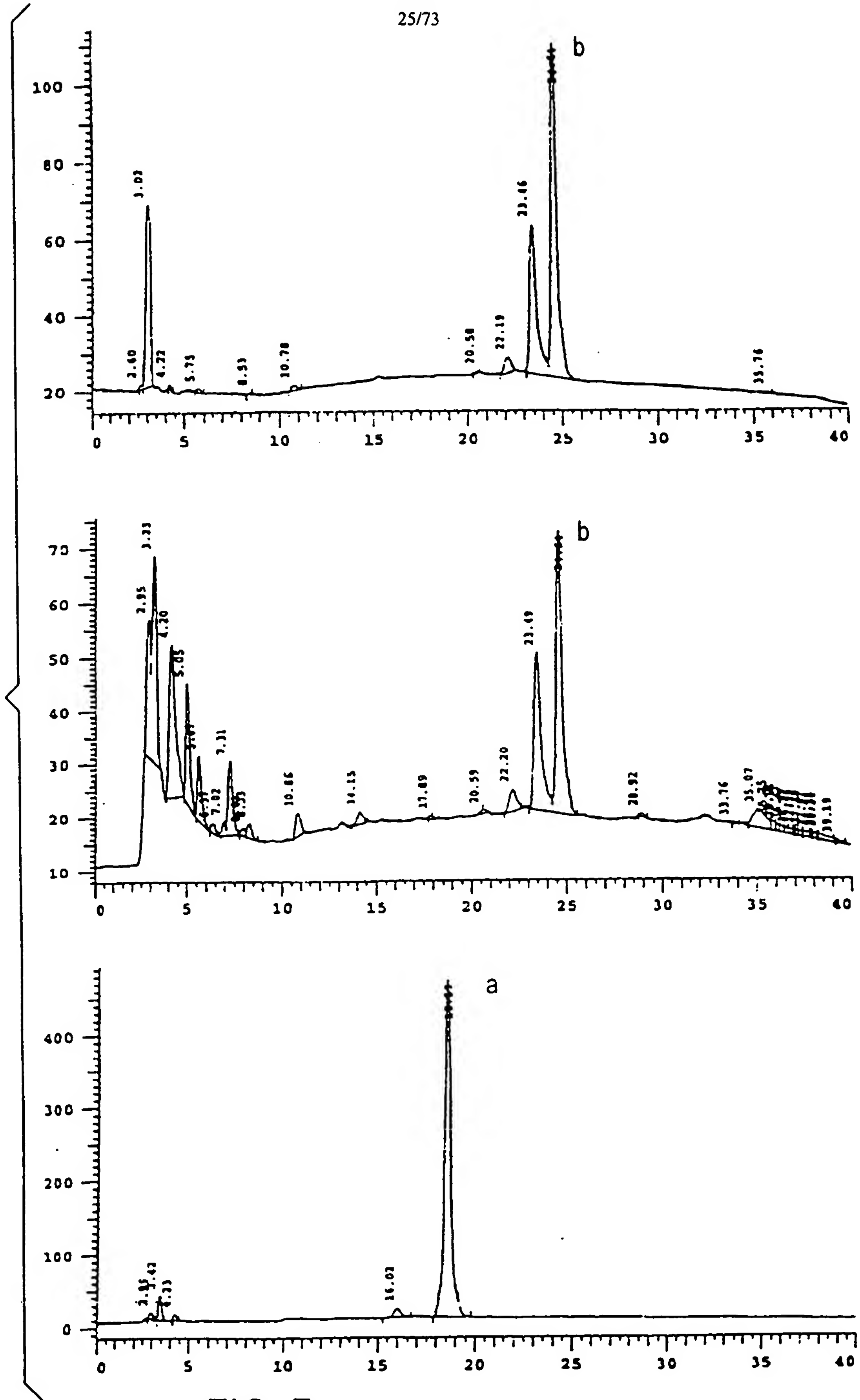


FIG. 7

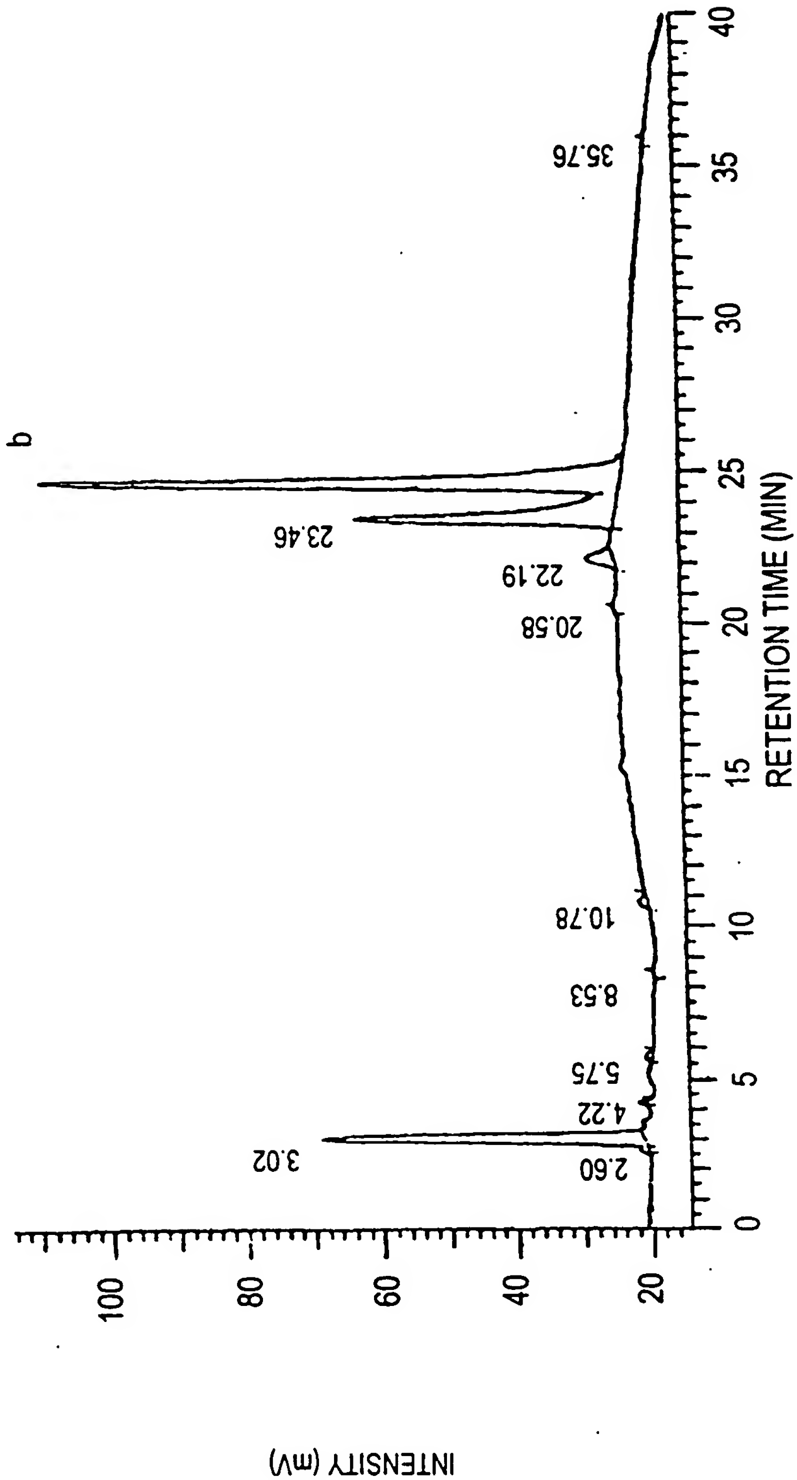

b
3.02
2.60
4.22
5.75
8.53
10.78
20.58
22.19
23.46
35.76
0
5
10
15
20
25
30
35
40
RETENTION TIME (MIN)
20
40
60
80
100
INTENSITY (mV)

FIG. 7A

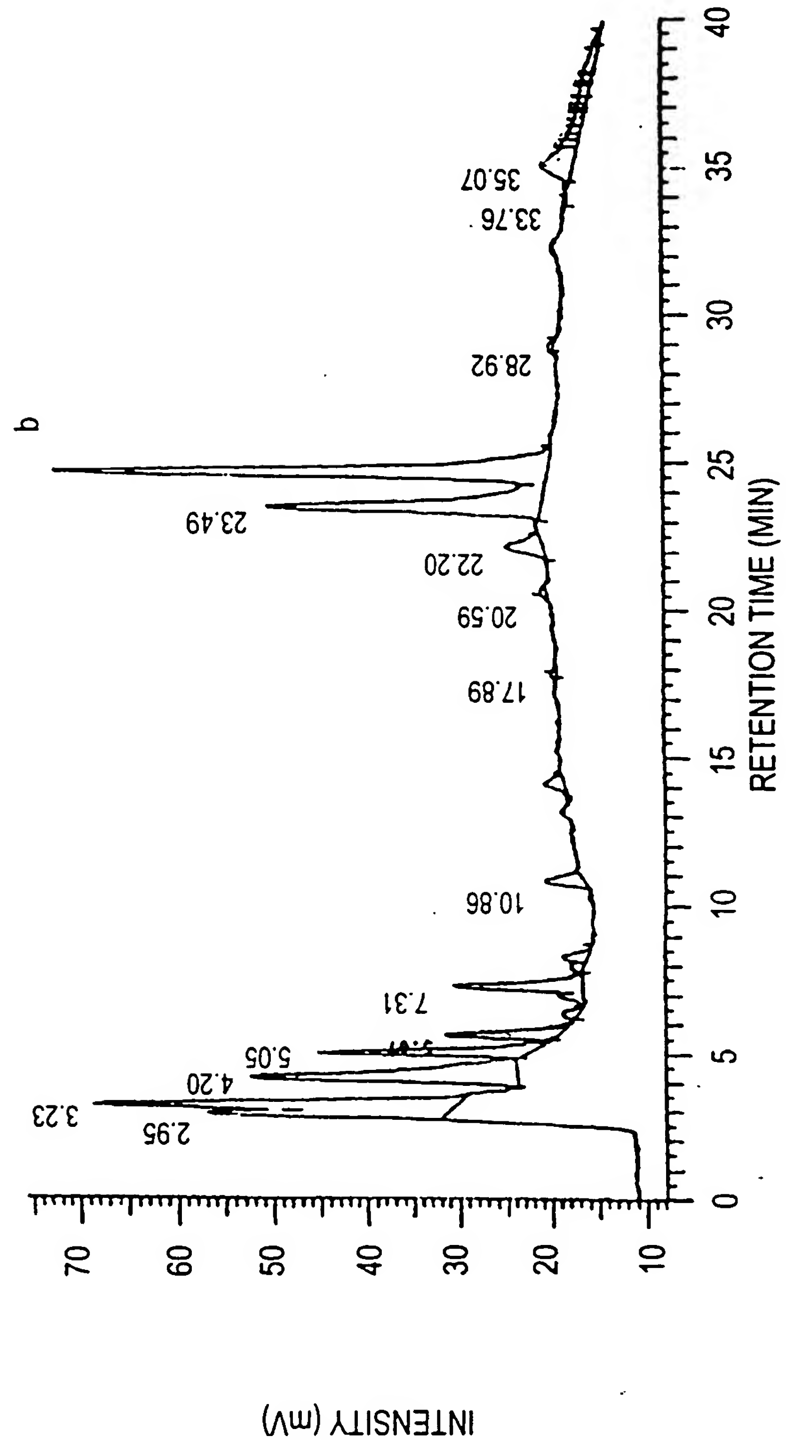
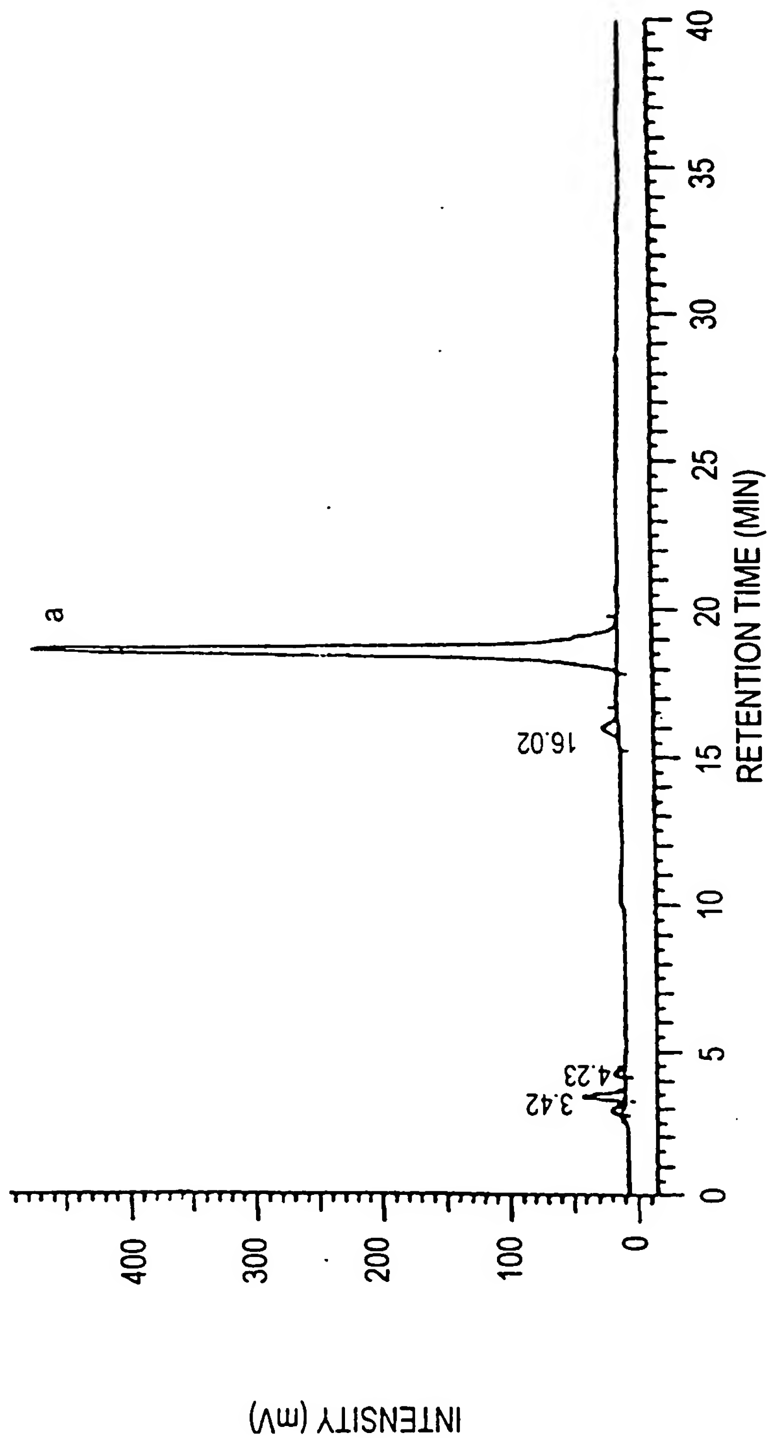

b
INTENSITY (mV)
RETENTION TIME (MIN)
2.95
3.23
4.20
5.05
7.31
10.86
17.89
20.59
22.20
23.49
28.92
33.76
35.07

FIG. 7B



INTENSITY (mV)
0
100
200
300
400
RETENTION TIME (MIN)
0
5
10
15
20
25
30
35
40
3.42
4.23
16.02
a

FIG. 7C

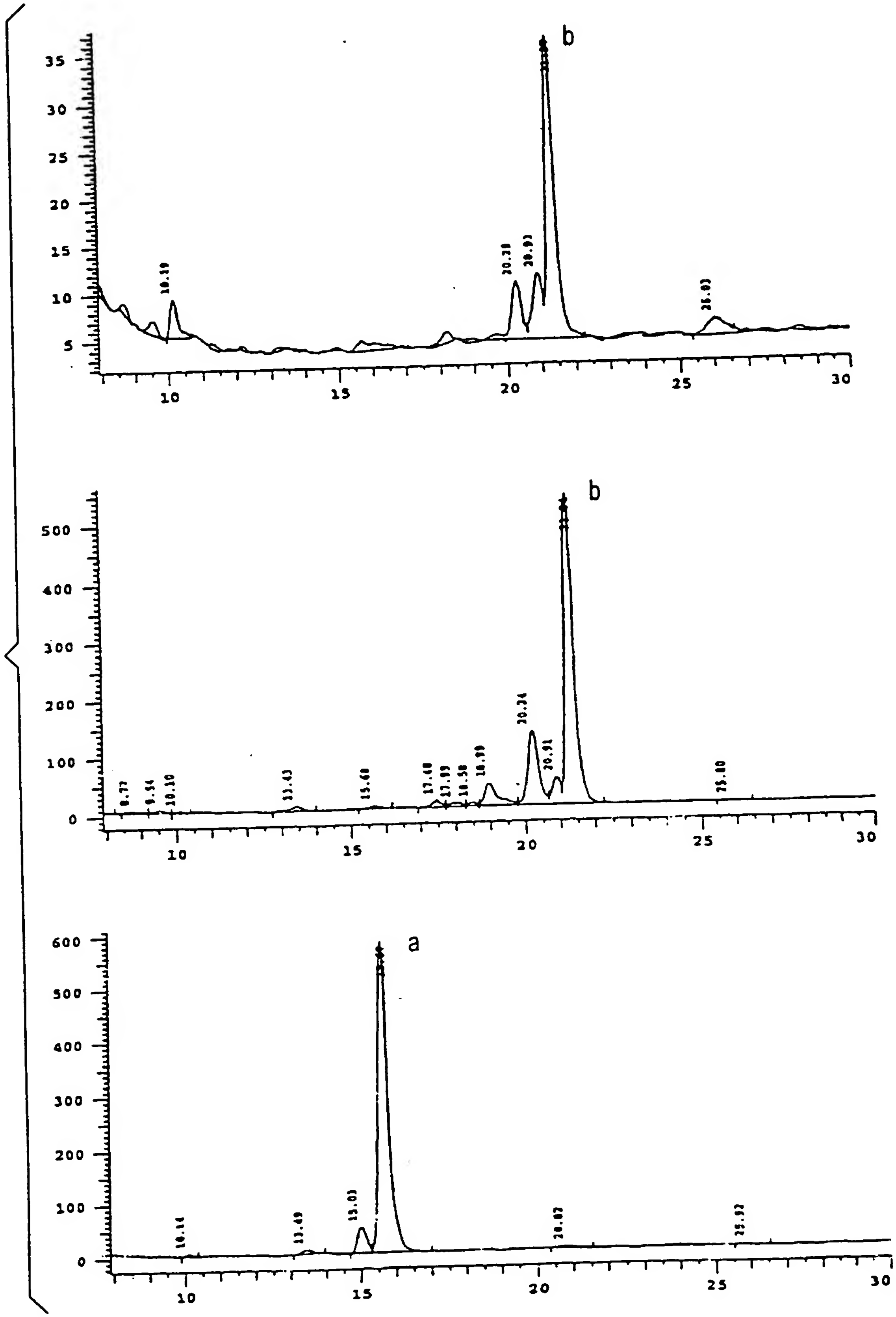

b
35
30
25
20
15
10
5
10
15
20
25
30
b
500
400
300
200
100
0
10
15
20
25
30
a
600
500
400
300
200
100
0
10
15
20
25
30

FIG. 8

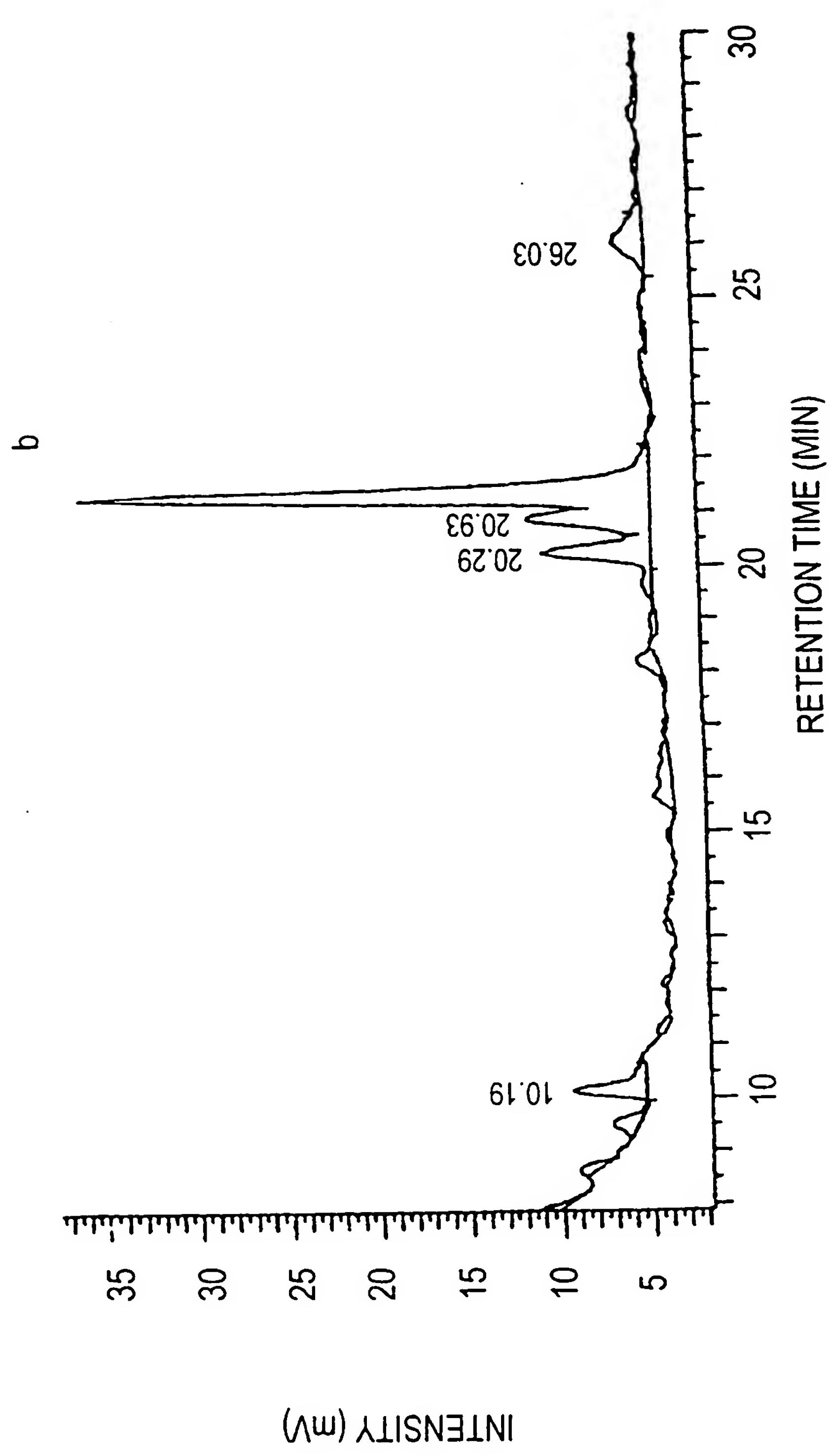

b
26.03
20.93
20.29
10.19
35
30
25
20
15
10
5
INTENSITY (mV)
10
15
20
25
30
RETENTION TIME (MIN)

FIG. 8A

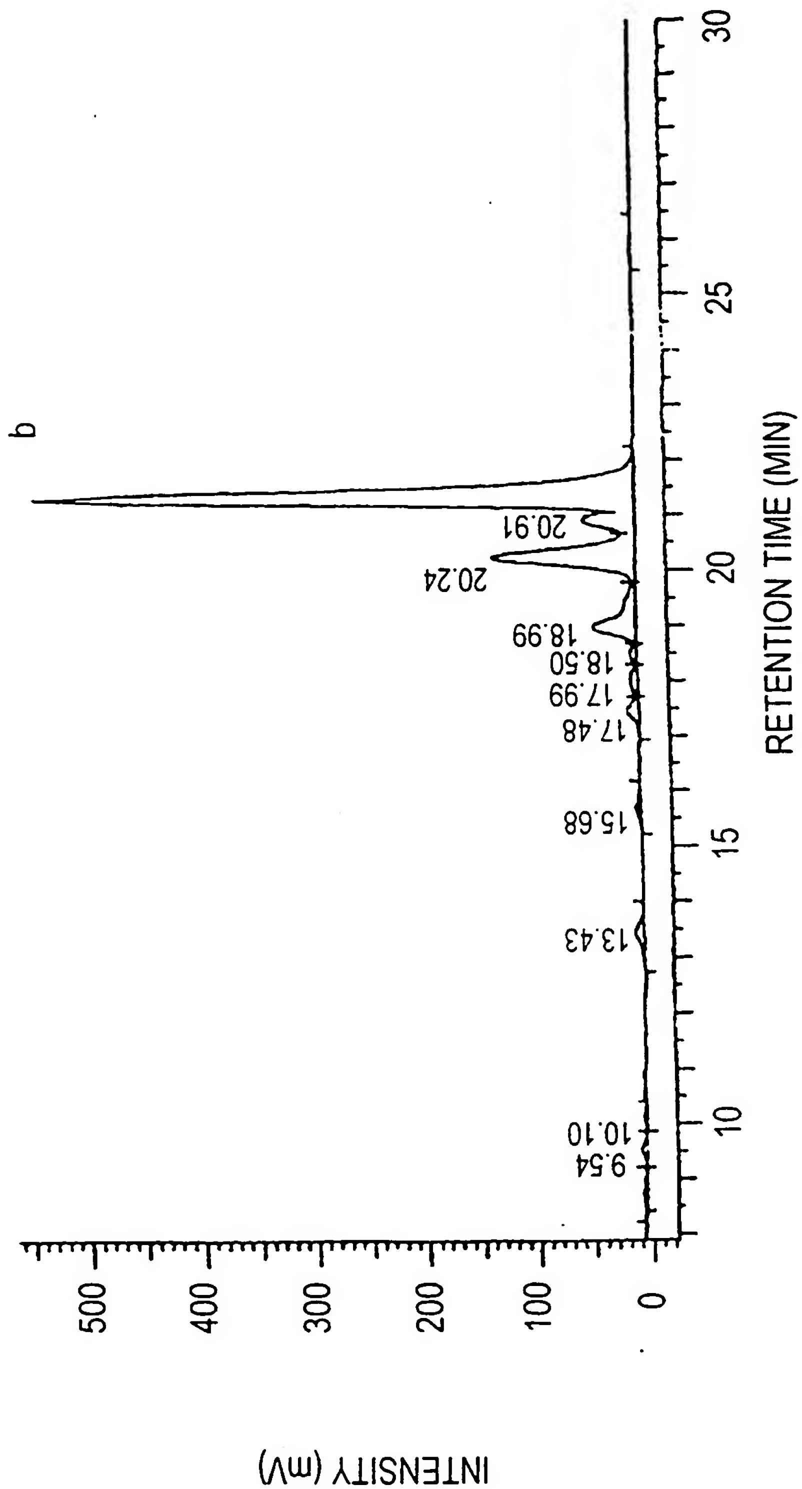

b
20.91
20.24
18.99
18.50
17.99
17.48
15.68
13.43
10.10
9.54
10
15
20
25
30
RETENTION TIME (MIN)
0
100
200
300
400
500
INTENSITY (mV)

FIG. 8B

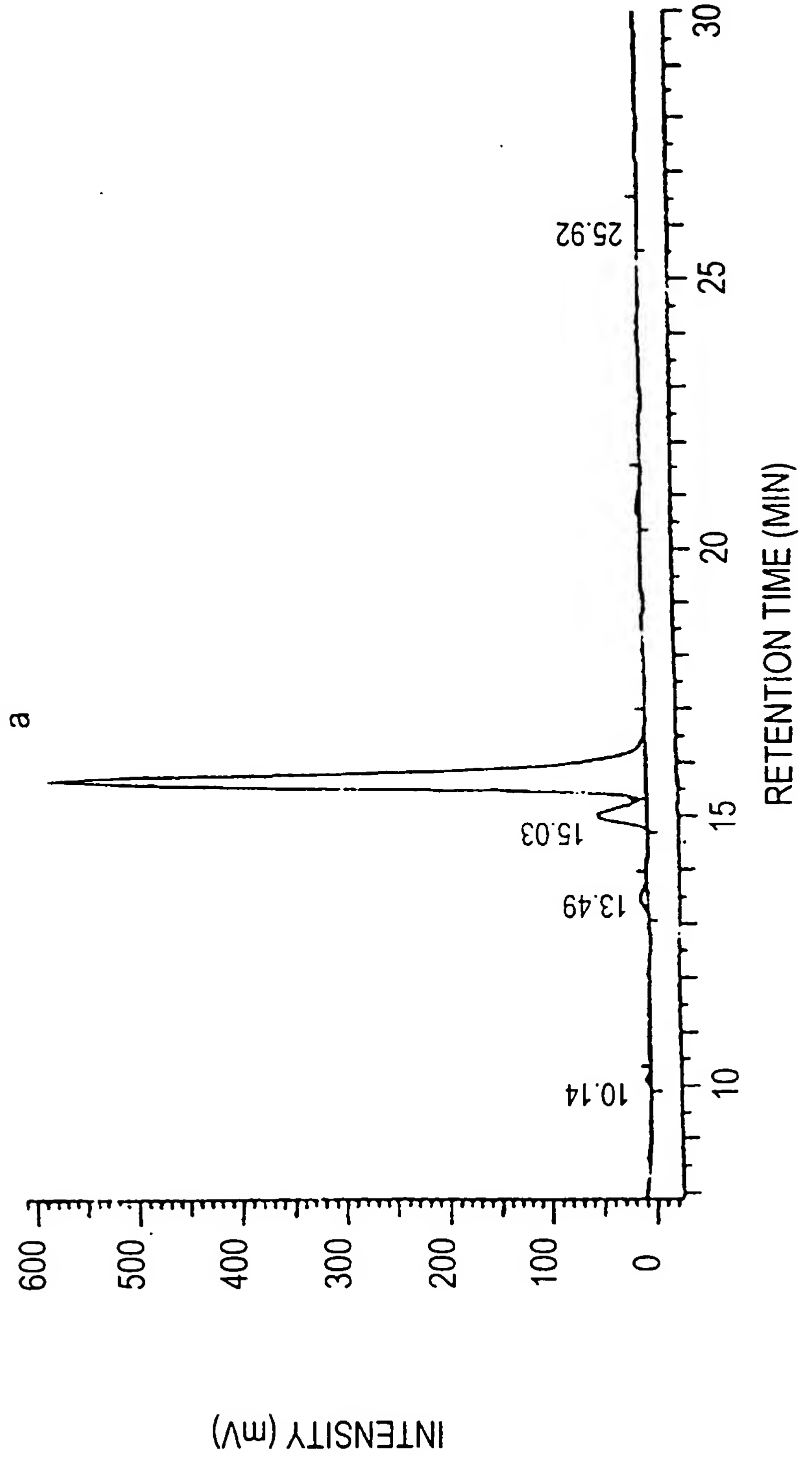

a
10.14
13.49
15.03
25.92
600
500
400
300
200
100
0
INTENSITY (mV)
10
15
20
25
30
RETENTION TIME (MIN)

FIG. 8C

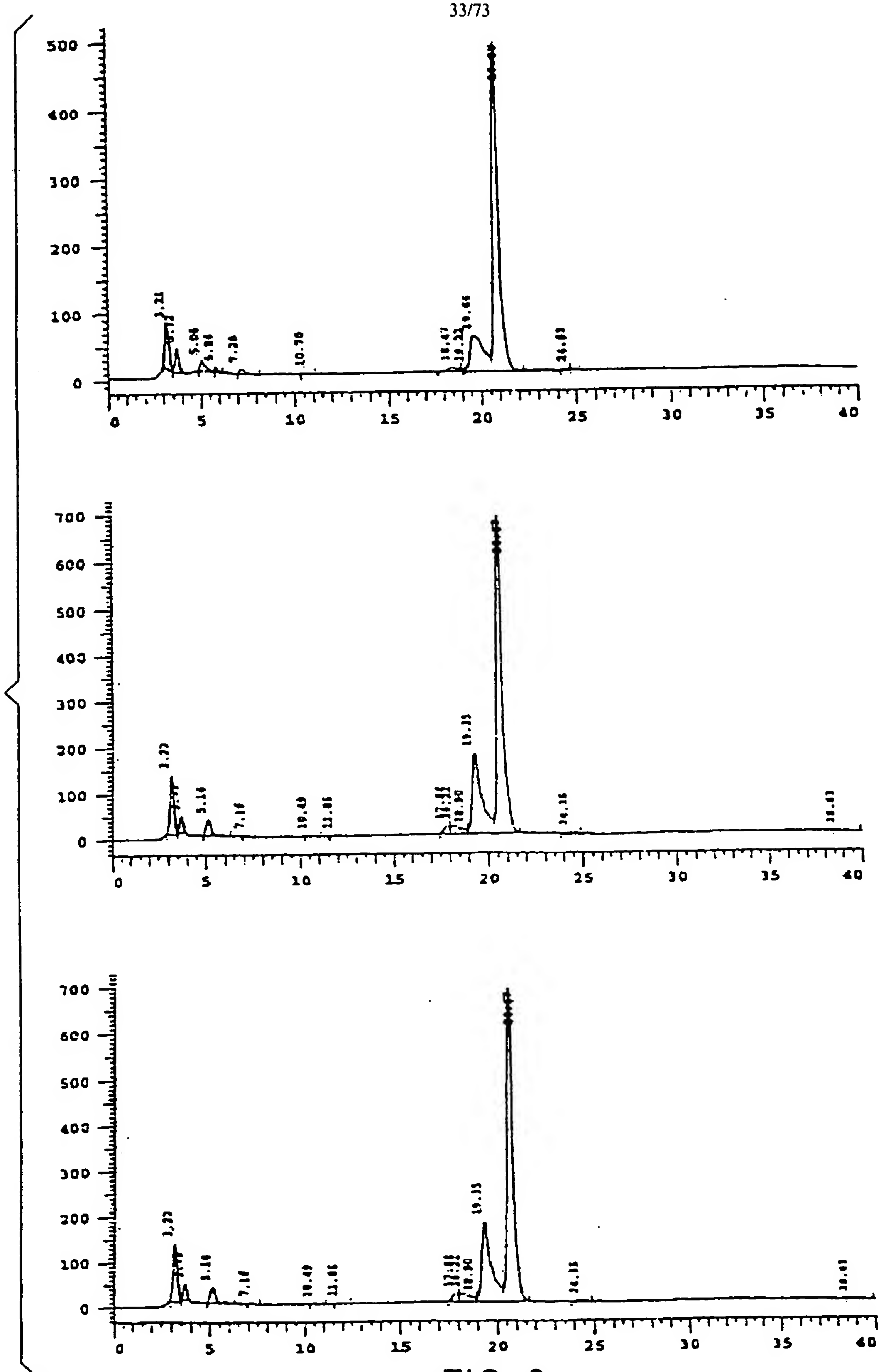


FIG. 9

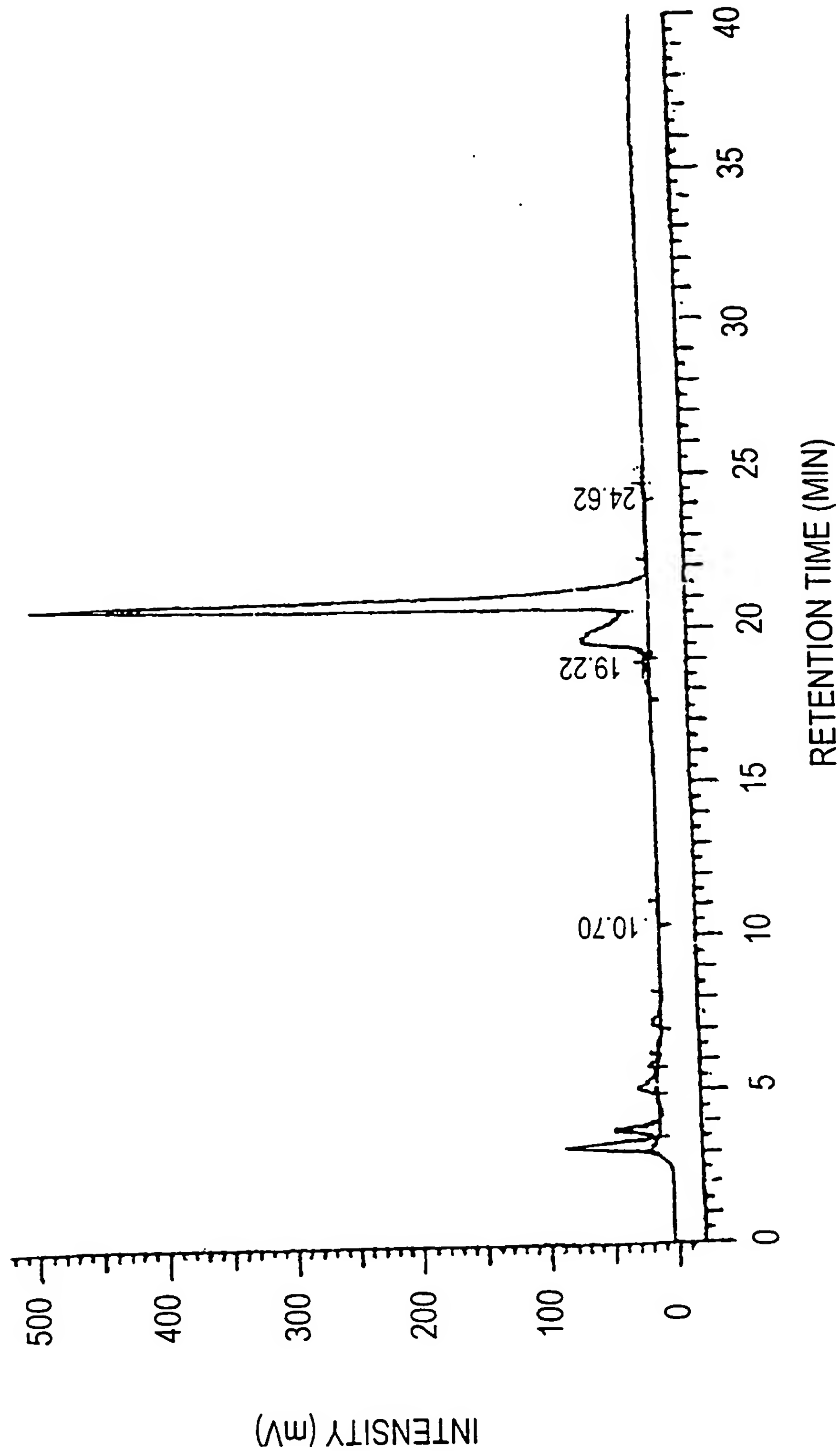

24.62
19.22
10.70
0
5
10
15
20
25
30
35
40
RETENTION TIME (MIN)
0
100
200
300
400
500
INTENSITY (mV)

FIG. 9A

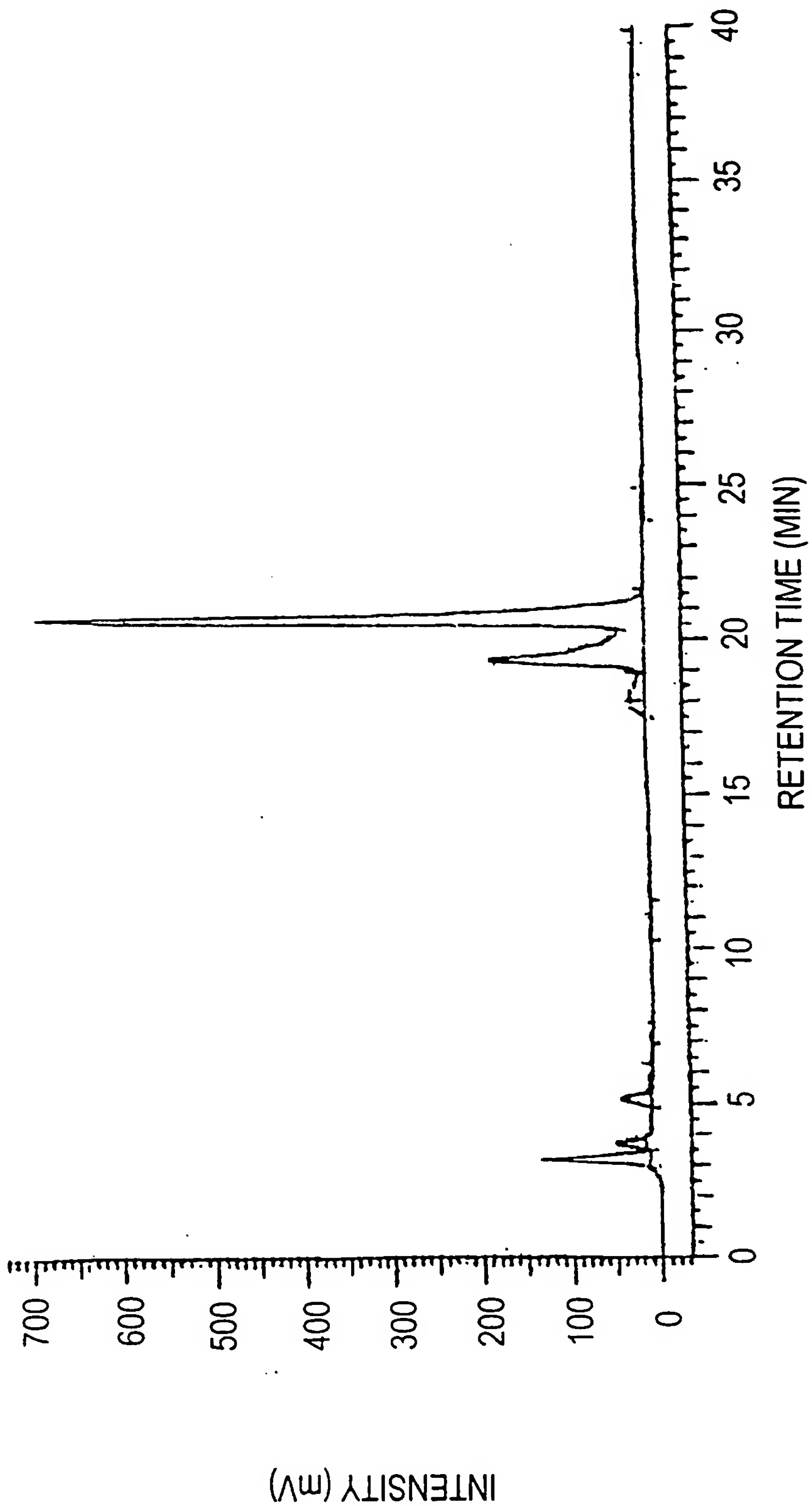

INTENSITY (mV)
0
100
200
300
400
500
600
700
RETENTION TIME (MIN)
0
5
10
15
20
25
30
35
40

FIG. 9B

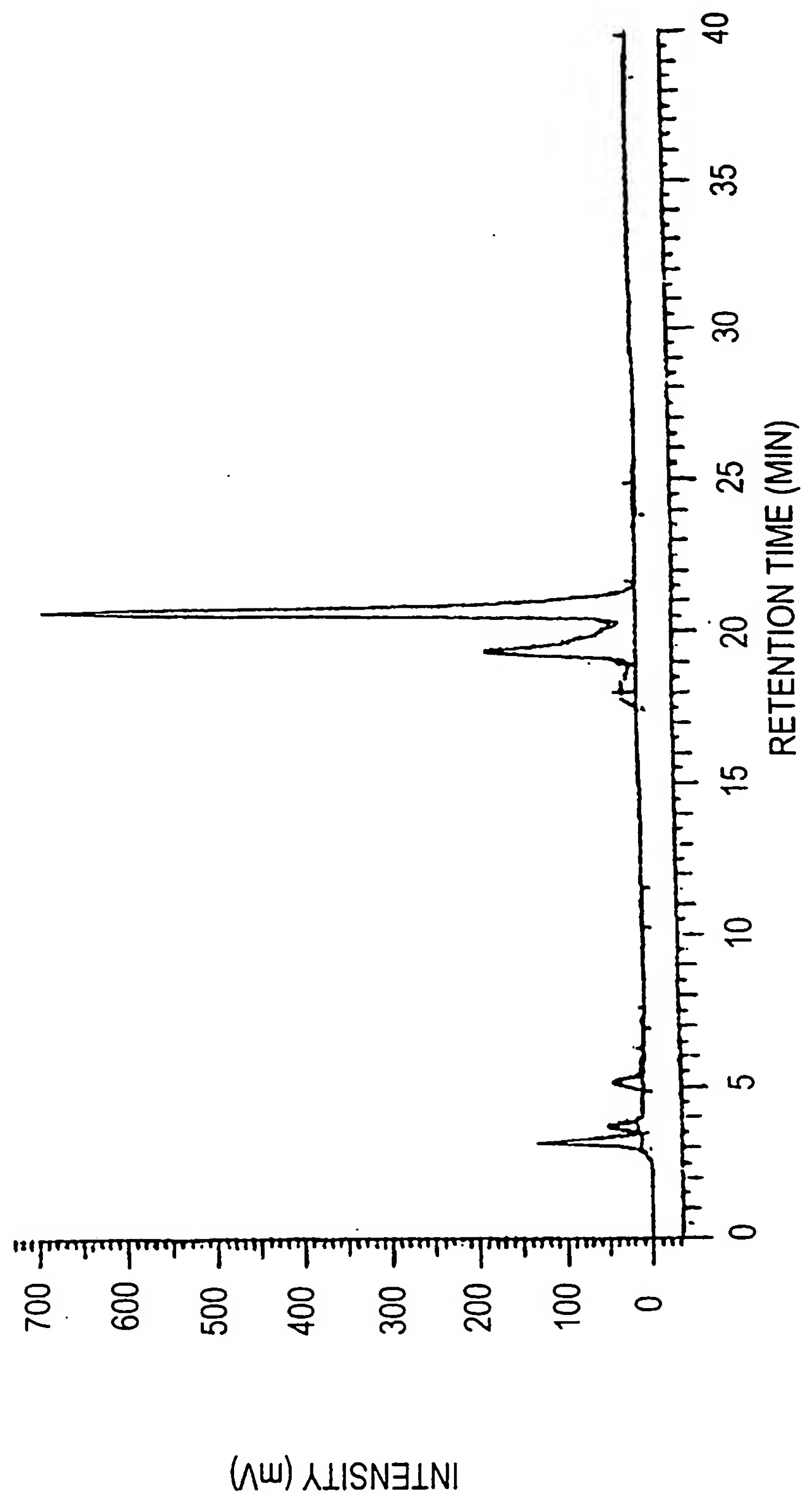

INTENSITY (mV)
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
RETENTION TIME (MIN)

FIG. 9C

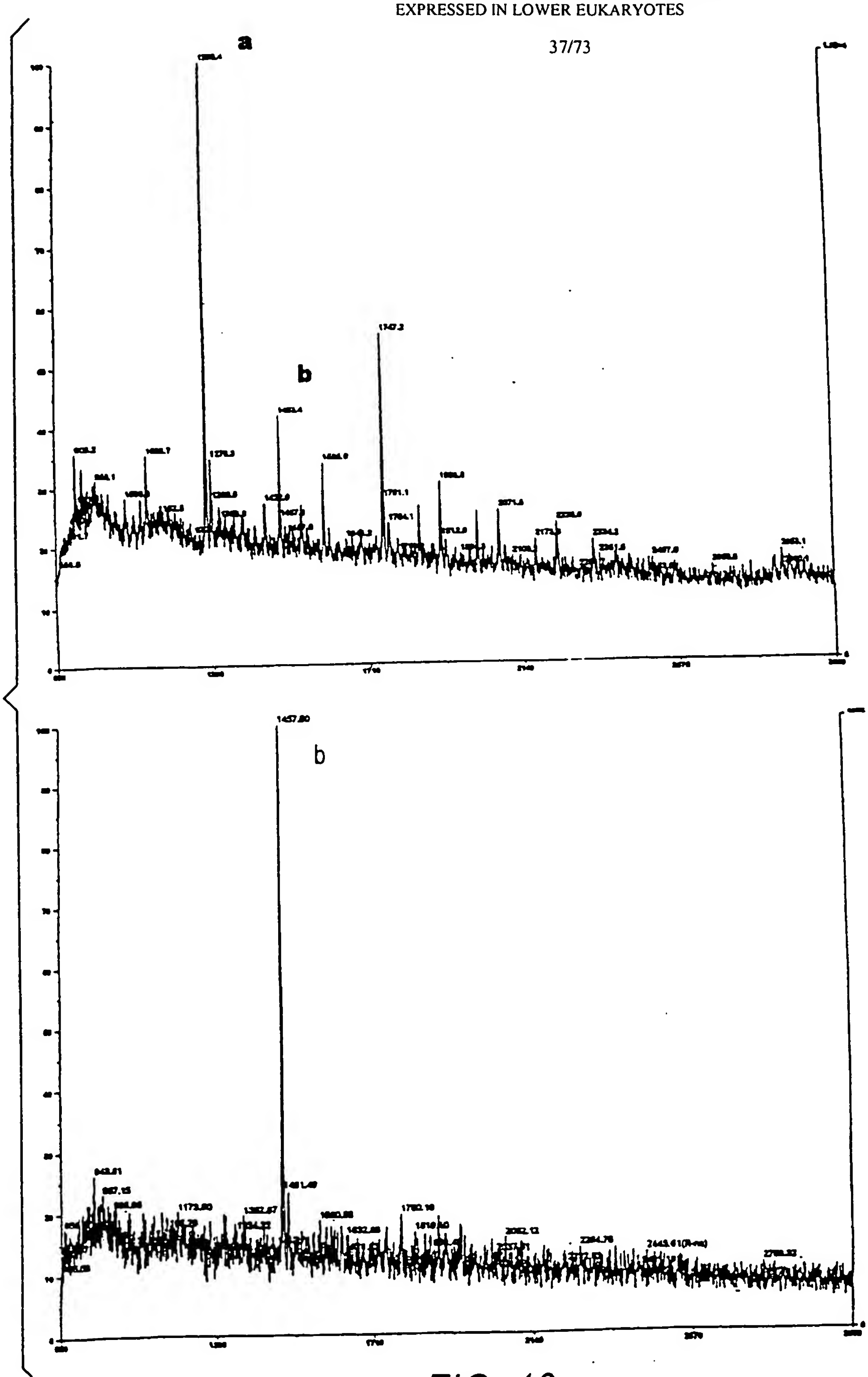

a
b
1747.3
b
1457.80
943.61
857.15
1173.80
1362.67
1461.49
1660.58
1632.86
1780.16
1819.40
2062.12
2264.76
2443.61(M-Na)
2798.32

FIG. 10

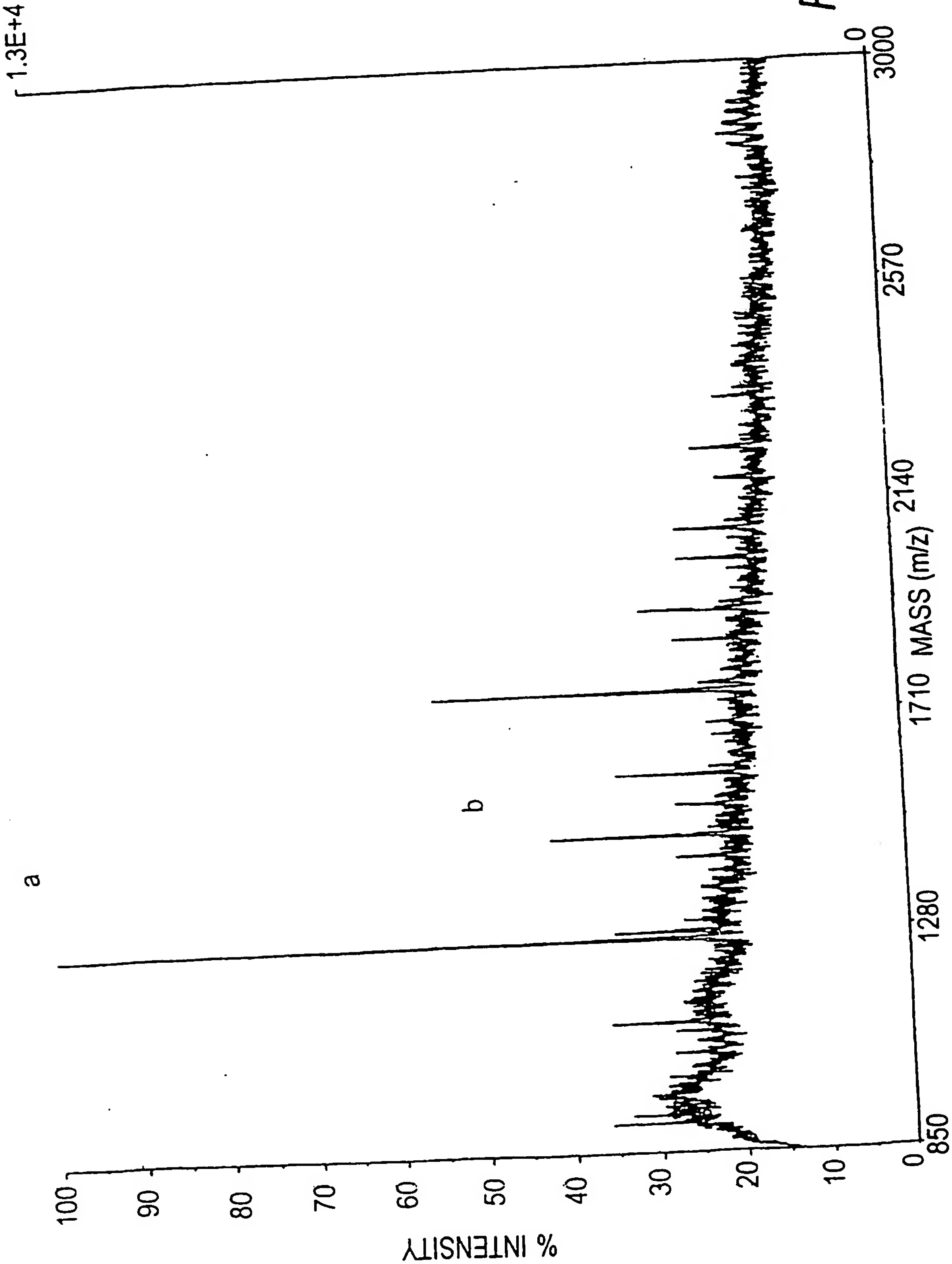

1.3E+4
a
b
% INTENSITY
100
90
80
70
60
50
40
30
20
10
0
850
1280
1710
2140
2570
3000
MASS (m/z)

FIG. 10A

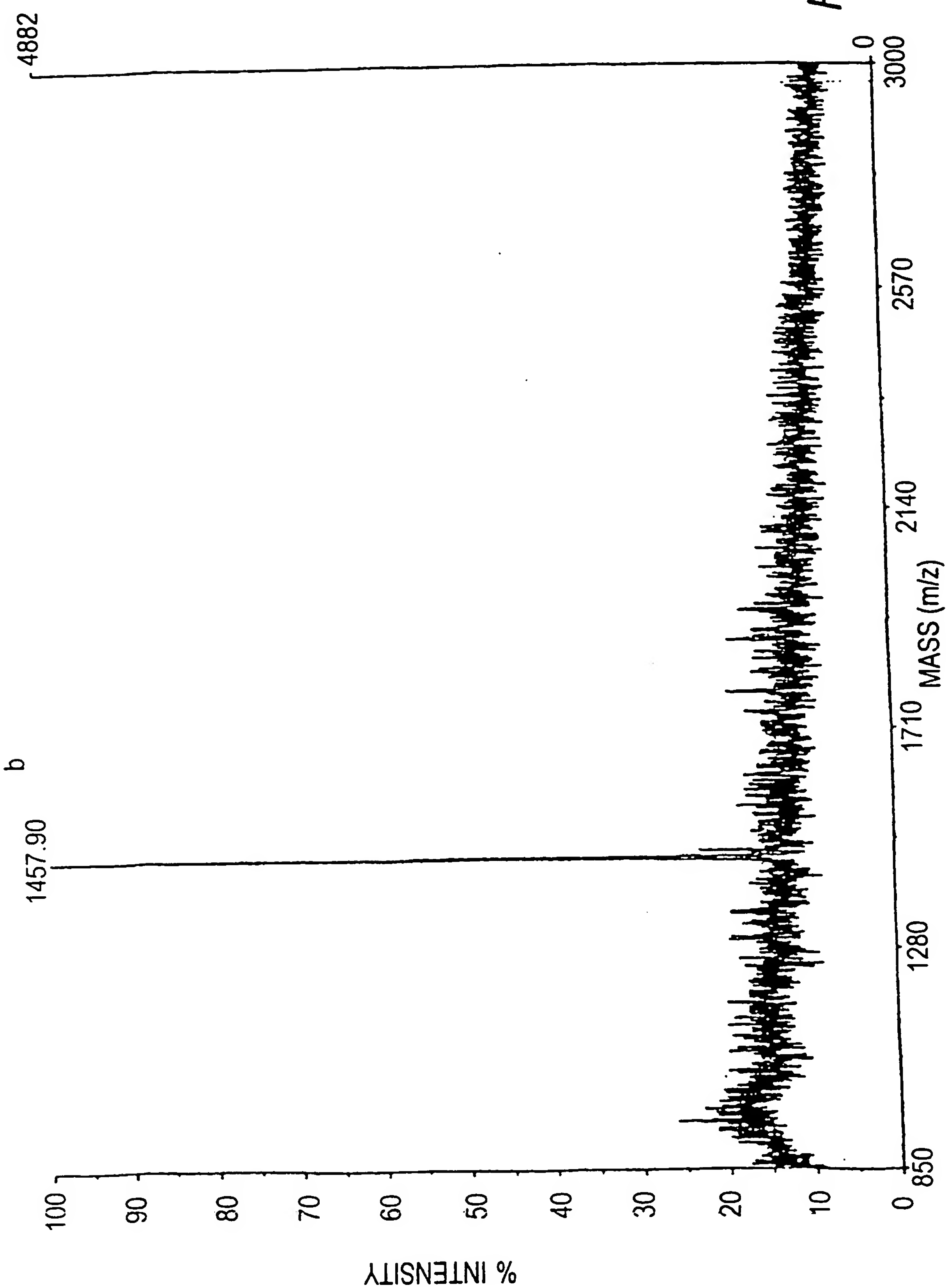
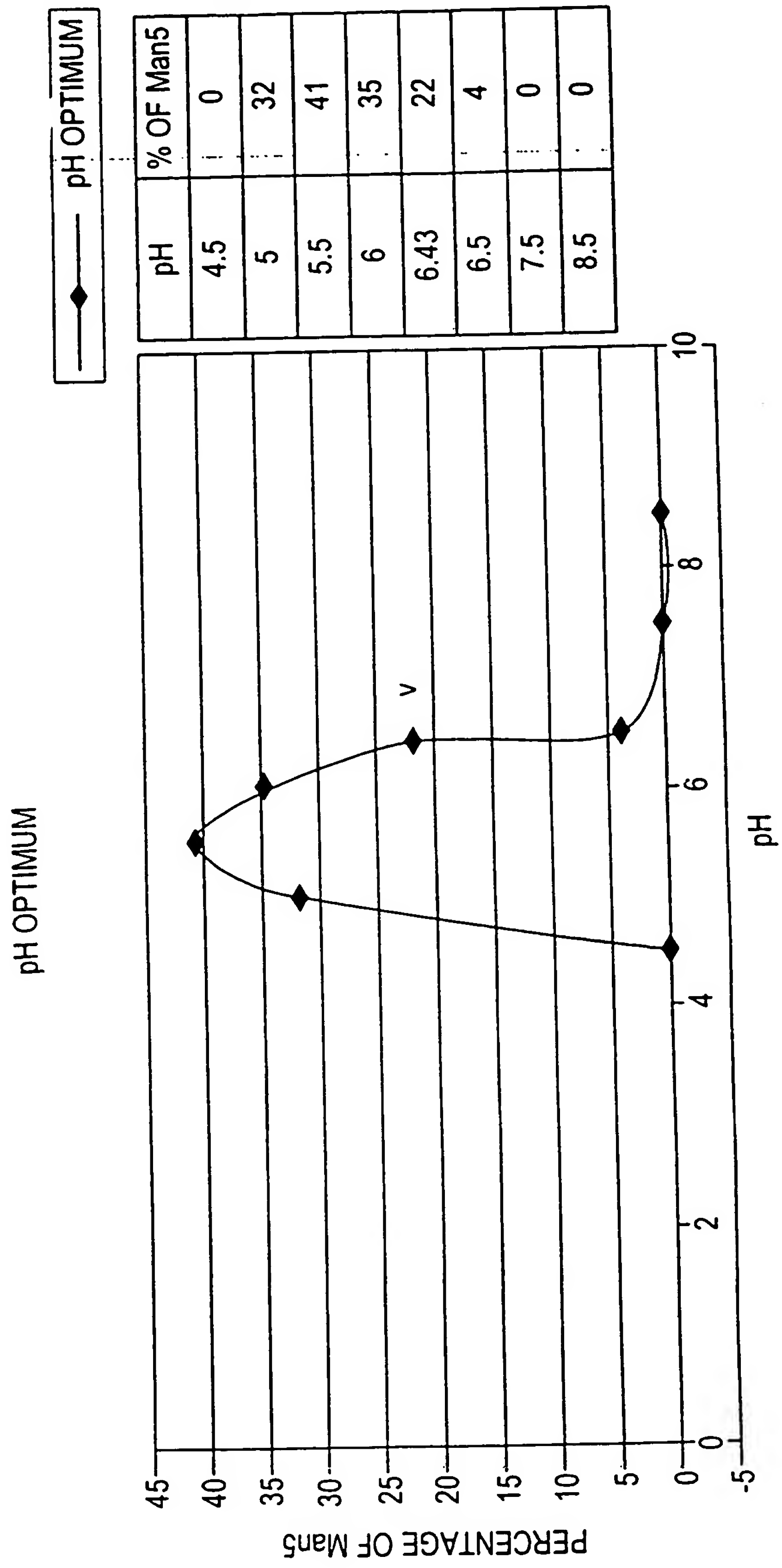

b
1457.90
4882
% INTENSITY
MASS (m/z)
100
90
80
70
60
50
40
30
20
10
0
850
1280
1710
2140
2570
3000

FIG. 10B



pH	% OF Man5
4.5	0
5	32
5.5	41
6	35
6.43	22
6.5	4
7.5	0
8.5	0

FIG. 11

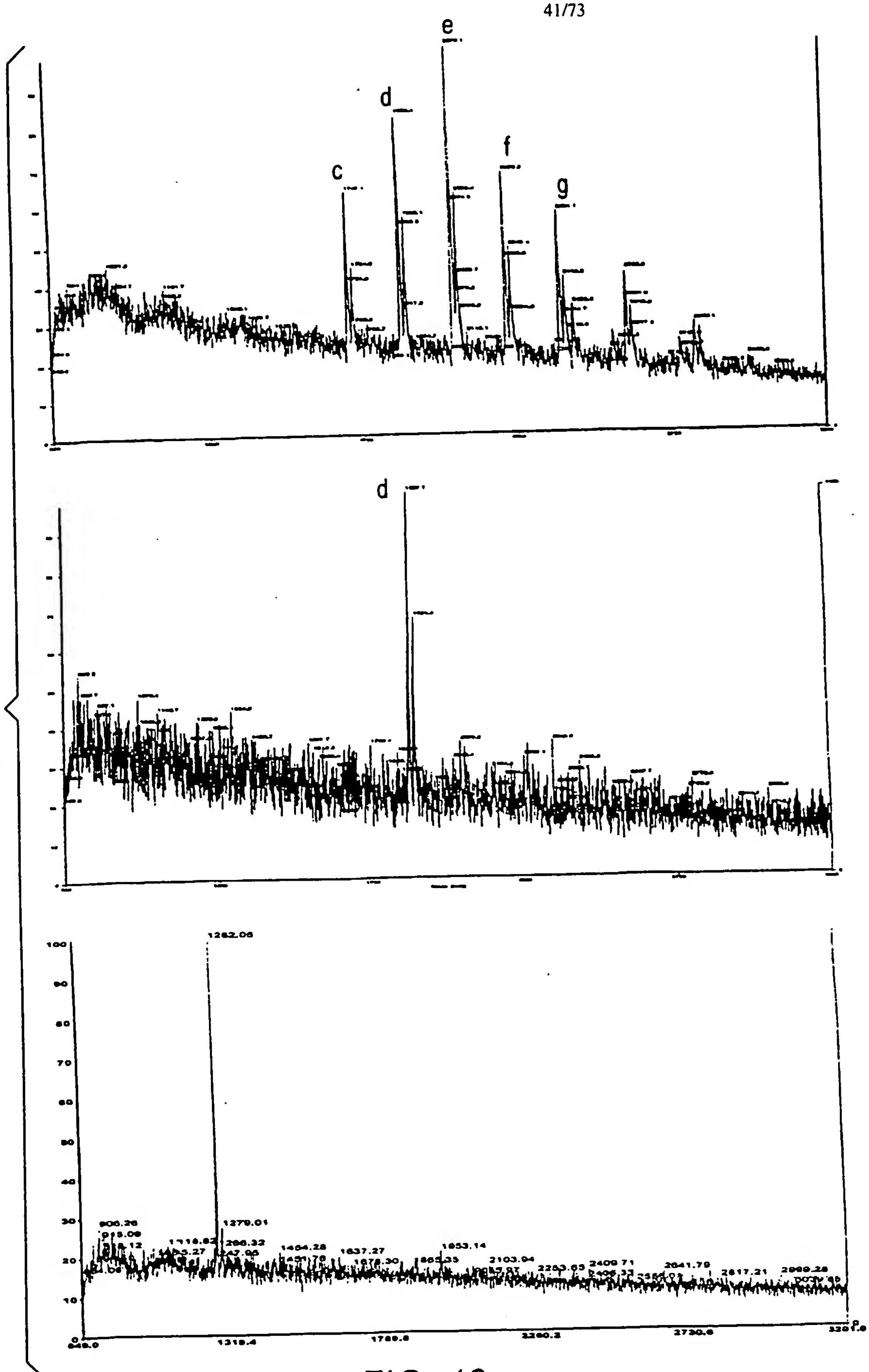
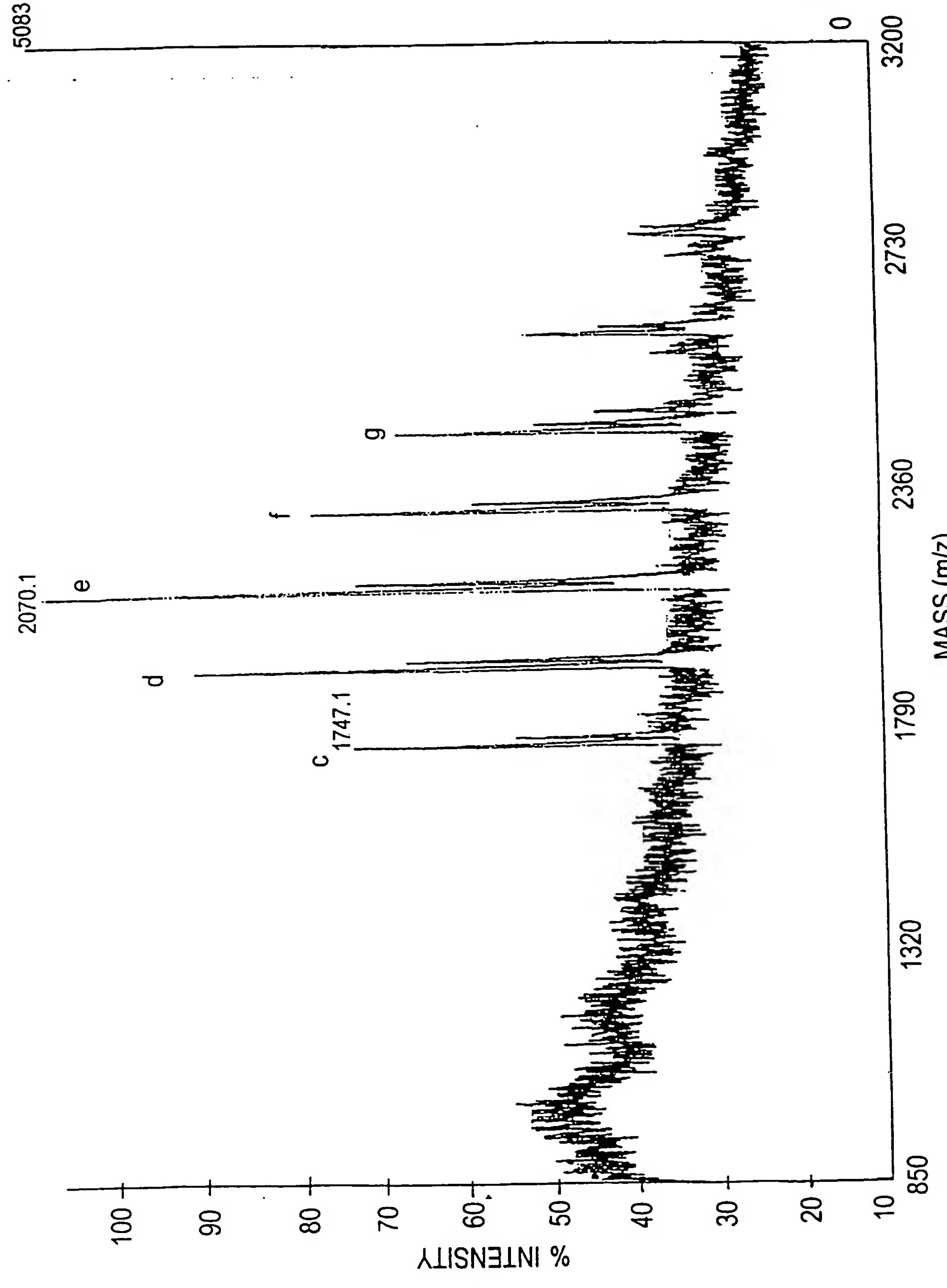

c
d
e
f
g
d
1262.06
1279.01
1653.14
1637.27
1464.28
2103.04
2409.71
2641.79
2617.21
906.26
1318.4
1789.8
2730.8

FIG. 12



5083
2070.1
e
d
1747.1
c
f
g
0
% INTENSITY
100
90
80
70
60
50
40
30
20
10
850
1320
1790
2360
2730
3200
MASS (m/z)

FIG. 12A

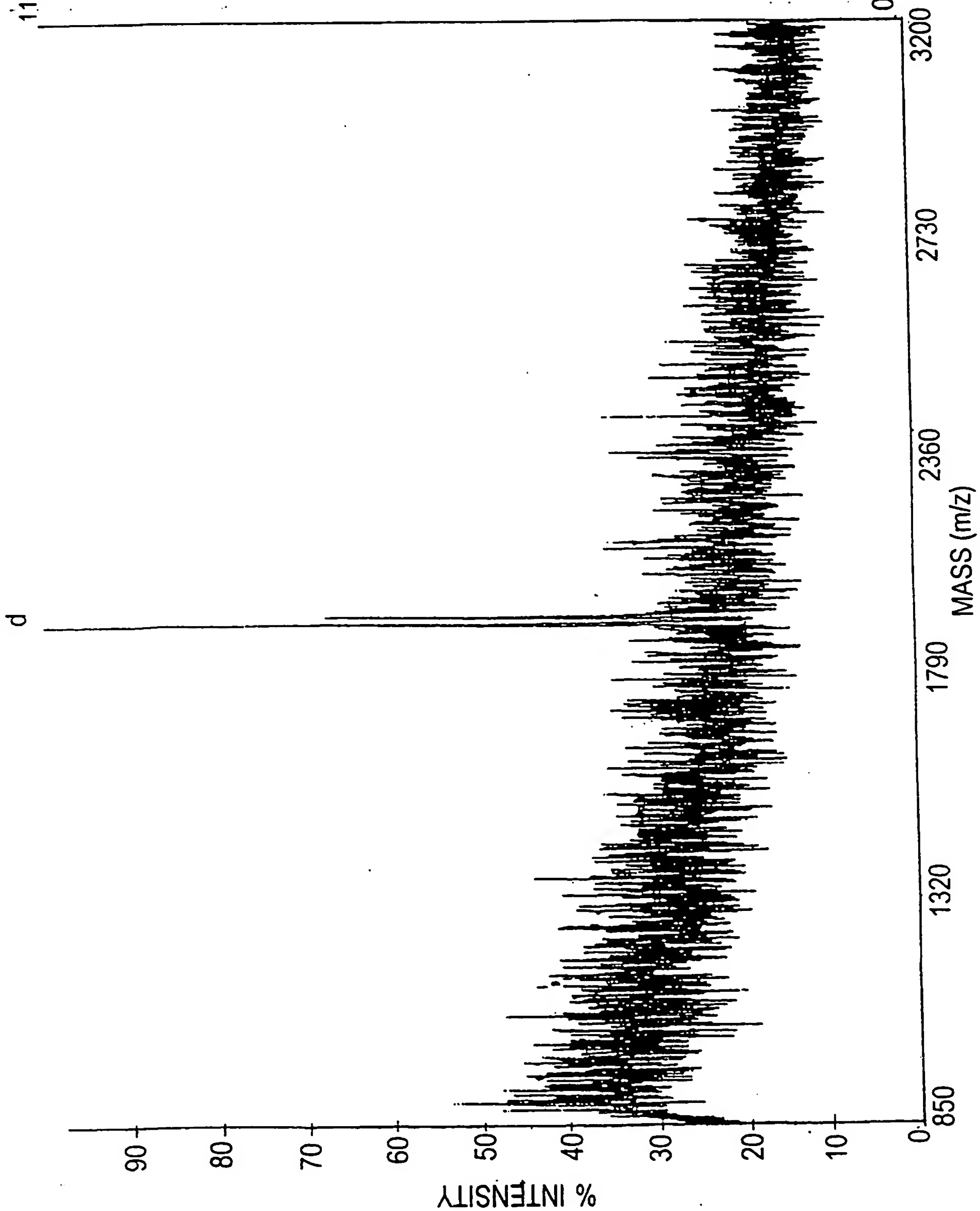
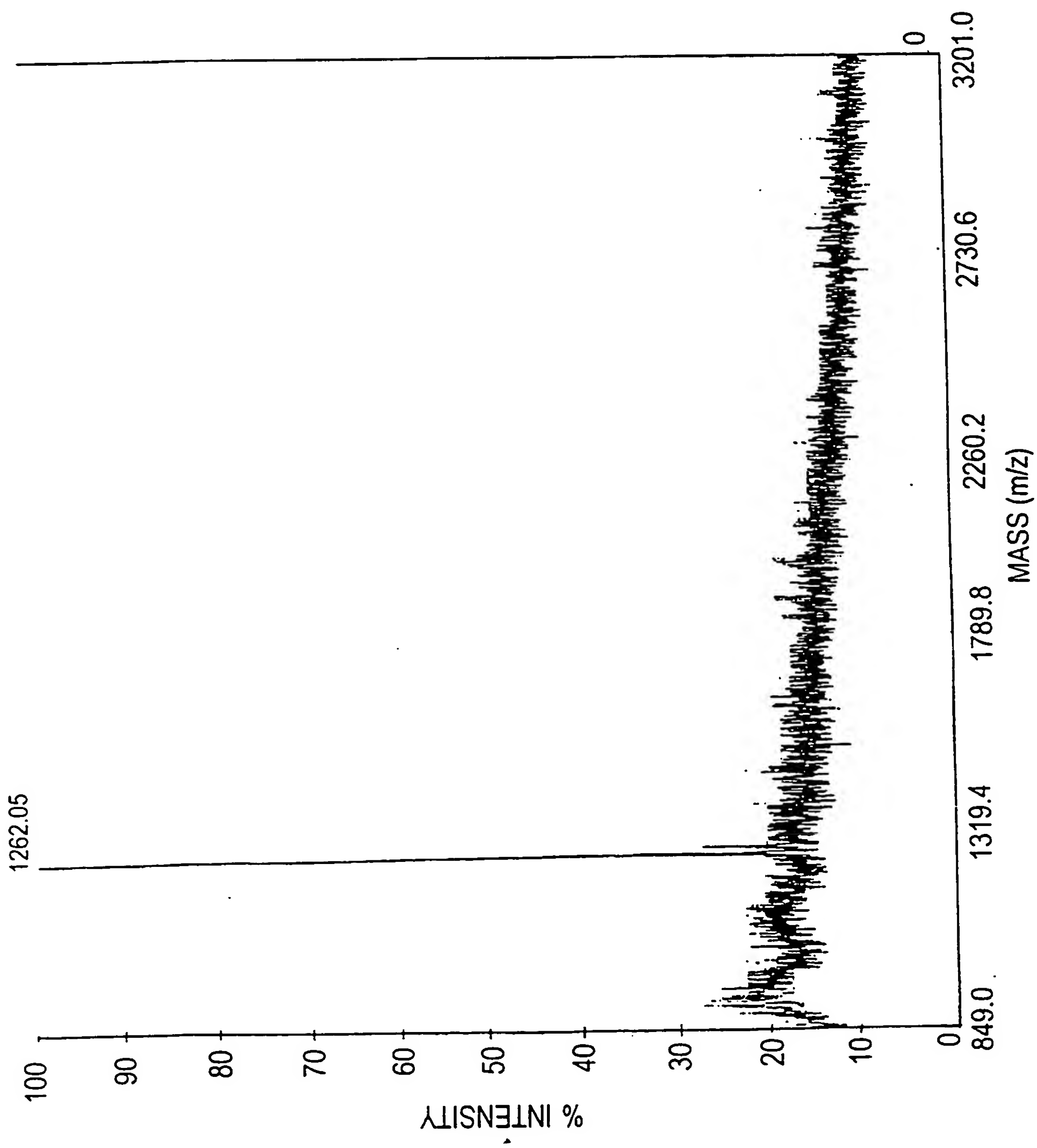

1128
d
% INTENSITY
0
10
20
30
40
50
60
70
80
90
MASS (m/z)
850
1320
1790
2360
2730
3200

FIG. 12B

FIG. 12C


1262.05
% INTENSITY
100
90
80
70
60
50
40
30
20
10
0
849.0
1319.4
1789.8
2260.2
2730.6
3201.0
0
MASS (m/z)

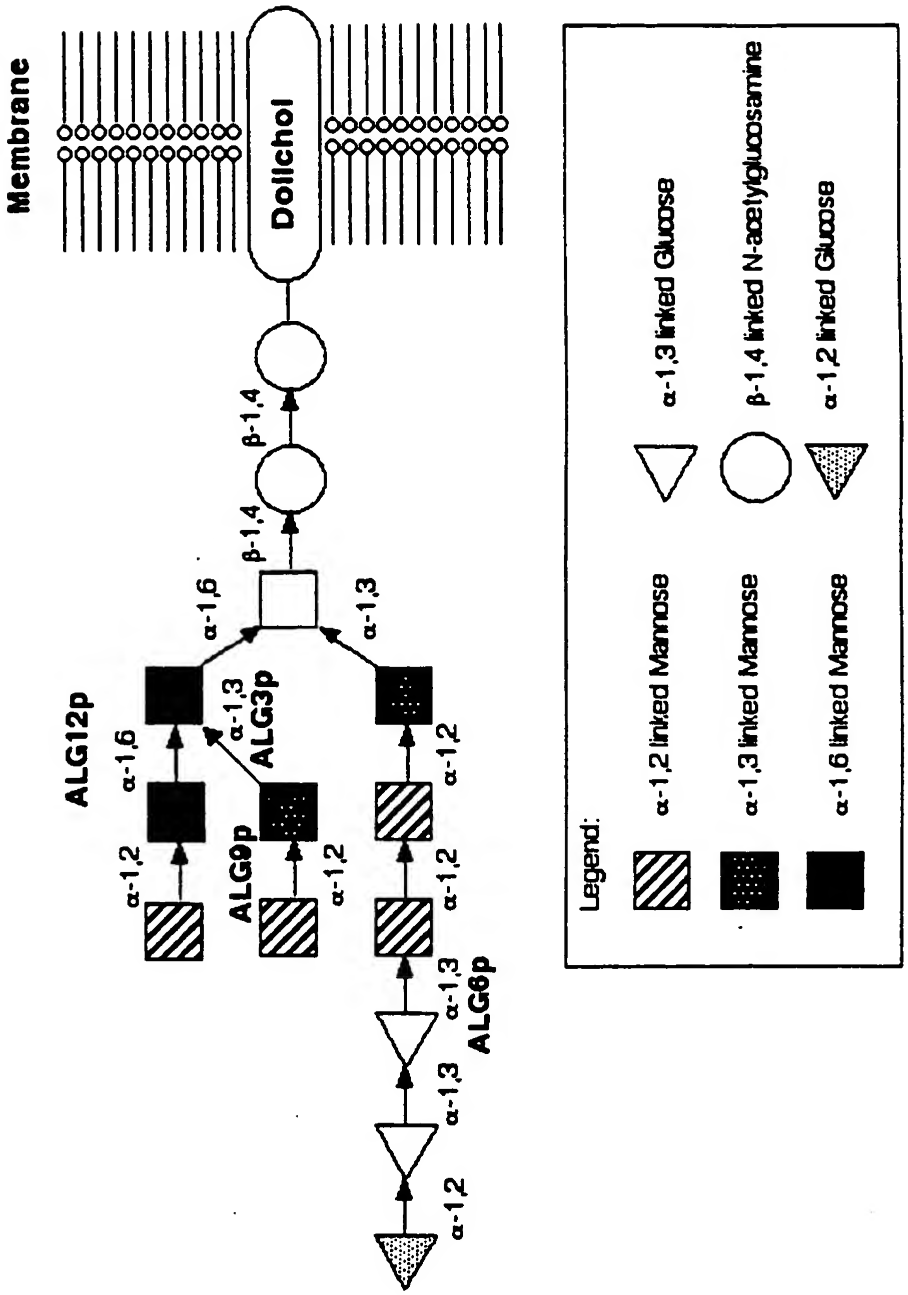

Membrane
Dolichol
ALG12p
ALG9p
ALG3p
ALG6p
α-1,2
α-1,6
α-1,3
β-1,4
Legend:
α-1,2 linked Mannose
α-1,3 linked Mannose
α-1,6 linked Mannose
α-1,3 linked Glucose
β-1,4 linked N-acetylglucosamine
α-1,2 linked Glucose

FIG. 13

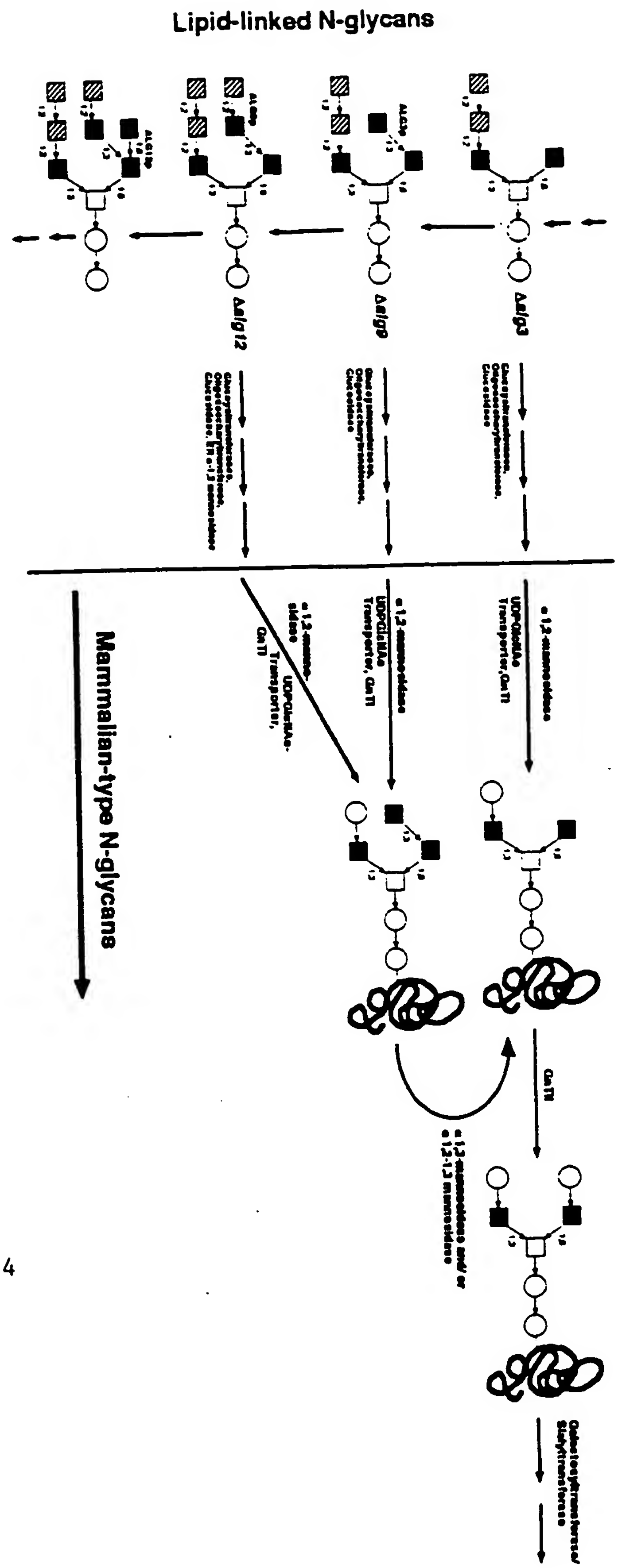

Lipid-linked N-glycans
Δalg12
Δalg9
Δalg3
Mammalian-type N-glycans
GnTII

FIG. 14

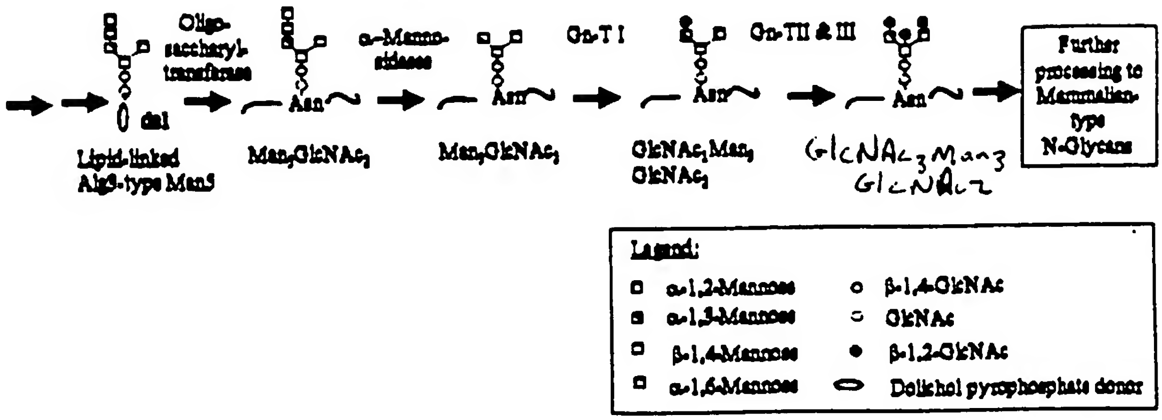

Oligo-saccharyl-transferase
α-Mannosidase
Gn-T I
Gn-TII & III
dol
Asn
Lipid-linked Alg3-type Man5
Man5GlcNAc2
Man3GlcNAc2
GlcNAc1Man3GlcNAc2
GlcNAc3Man3GlcNAc2
Further processing to Mammalian-type N-Glycans
Legend:
α-1,2-Mannose
α-1,3-Mannose
β-1,4-Mannose
α-1,6-Mannose
β-1,4-GlcNAc
GlcNAc
β-1,2-GlcNAc
Dolichol pyrophosphate donor

FIG. 15

ALG3 Blast 05-22-01

```
                                                                 (bits)  Value
Sequences producing significant alignments:

gi|586444|sp|P38179|ALG3_YEAST  DOLICHYL-P-MAN:MAN(5)GLCNAC(...797   0.0
gi|3024226|sp|Q92685|ALG3_HUMAN  DOLICHYL-P-MAN:MAN(5)GLCNAC...173   7e-43
gi|3024221|sp|Q24332|NT56_DROVI  LETHAL(2)NEIGHBOUR OF TID P...145   3e-34
gi|3024222|sp|Q27333|NT56_DROME  LETHAL(2)NEIGHBOUR OF TID P...121   3e-27
gi|10720153|sp|P82149|NT53_DROME  LETHAL(2)NEIGHBOUR OF TID ...121   5e-27
gi|1707982|sp|P40989|GLS2_YEAST  1,3-BETA-GLUCAN SYNTHASE CO... 32   2.8
gi|1346146|sp|P38631|GLS1_YEAST  1,3-BETA-GLUCAN SYNTHASE CO... 31   6.6
```

Alignments

Yeast

>gi|586444|sp|P38179|ALG3_YEAST DOLICHYL-P-MAN:MAN(5)GLCNAC(2)-PP-DOLICHYL MANNOSYLTRANSFERASE

```
          (DOL-P-MAN DEPENDENT ALPHA(1-3)-MANNOSYLTRANSFERASE)
          (HM-1 KILLER TOXIN RESISTANCE PROTEIN)
          Length = 458

 Score =  797 bits (2059), Expect = 0.0
 Identities = 422/458 (92%), Positives = 422/458 (92%)

Query: 1    MEGEQSPQGEKSLQRKQFVRPPLDLWQDLKDGVRYVIFDCRANLIVMPLLILFESMLCKI 60
            MEGEQSPQGEKSLQRKQFVRPPLDLWQDLKDGVRYVIFDCRANLIVMPLLILFESMLCKI
Sbjct: 1    MEGEQSPQGEKSLQRKQFVRPPLDLWQDLKDGVRYVIFDCRANLIVMPLLILFESMLCKI 60

Query: 61   IIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGM 120
            IIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGM
Sbjct: 61   IIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGM 120

Query: 121  DHVERGQVFFRYLYLLTLALQMACYYLLHLPPWCVVLACLSKRLHSIYVLRLFNDCFTTL 180
            DHVERGQVFFRYLYLLTLALQMACYYLLHLPPWCVVLACLSKRLHSIYVLRLFNDCFTTL
Sbjct: 121  DHVERGQVFFRYLYLLTLALQMACYYLLHLPPWCVVLACLSKRLHSIYVLRLFNDCFTTL 180

Query: 181  FMVVTVLGAIVASRCHQRPKLKKSLALVISATYSMAVSIKMNALLYFPAMMISLFILNDA 240
            FMVVTVLGAIVASRCHQRPKLKKSLALVISATYSMAVSIKMNALLYFPAMMISLFILNDA
Sbjct: 181  FMVVTVLGAIVASRCHQRPKLKKSLALVISATYSMAVSIKMNALLYFPAMMISLFILNDA 240

Query: 241  NVILTLLDLVAMIAWQVAVAVPFLRSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFND 300
            NVILTLLDLVAMIAWQVAVAVPFLRSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFND
Sbjct: 241  NVILTLLDLVAMIAWQVAVAVPFLRSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFND 300

Query: 301  KRFXXXXXXXXXXXXXXXFVTRYPRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASN 360
            KRF               FVTRYPRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASN
Sbjct: 301  KRFHLALLISHLIALTTLFVTRYPRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASN 360

Query: 361  FIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYPPNSQXXXXX 420
            FIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYPPNSQ
Sbjct: 361  FIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYPPNSQASTLL 420

Query: 421  XXXXXXXXXXXXXXXXSGSVALAKSHLRTTSSMEKKLN 458
                            SGSVALAKSHLRTTSSMEKKLN
Sbjct: 421  LALNTVLLLLLALTQLSGSVALAKSHLRTTSSMEKKLN 458
```

FIG. 16-1

Human

```
>gi|3024226|sp|Q92685|ALG3_HUMAN DOLICHYL-P-MAN:MAN(5)GLCNAC(2)-PP-DOLICHYL
MANNOSYLTRANSFERASE
            (DOL-P-MAN DEPENDENT ALPHA(1-3)-MANNOSYLTRANSFERASE)
            (NOT56-LIKE PROTEIN)
          Length = 438

 Score =  173 bits (439), Expect = 7e-43
 Identities = 133/396 (33%), Positives = 195/396 (48%), Gaps = 28/396 (7%)

Query: 26  WQDLKDGVRYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLD 85
           WQ+     R ++ + R  L+V   L L E  +   +I +VAYTEID+KAYM ++E + ++
Sbjct: 29  WQER----RLLLREPRYTLLVAACLCLAEVGITFWVIHRVAYTEIDWKAYMAEVEGV-IN 83

Query: 86  GMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACY 145
           G  DY+Q+ G TGPLVYPAG V I+  +Y+ T     +   Q  F  LYL TL L    Y
Sbjct: 84  GTYDYTQLQGDTGPLVYPAGFVYIFMGLYYATSRGTDIRMAQNIFAVLYLATLLLVFLIY 143

Query: 146 Y-LLHLPPWC-VVLACLSKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKK 203
           +    +PP+    + C S R+HSI+VLRLFND      + + +L    +     QR
Sbjct: 144 HQTCKVPPFVFFFMCCASYRVHSIFVLRLFNDP-----VAMVLLFLSINLLLAQRWGWG- 197

Query: 204 SLALVISATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVAVPF 263
                    +S+AVS+KMN LL+ P ++  L         L  L + A + QV + +PF
Sbjct: 198 ------CCFFSLAVSVKMNVLLFAPGLLFLLLTQFGFRGALPKLGICAGL--QVVLGLPF 249

Query: 264 LRSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFNDKRFXXXXXXXXXXXXXXXFVTRY 323
           L   P  YL  +F+ GR+F++ W++NW+ + E  F  + F                + R+
Sbjct: 250 LLENPSGYLSRSFDLGRQFLFHWTVNWRFLPEALFLHRAFHLALLTAHLTLLLLFALCRW 309

Query: 324 PRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASNFIGVLFSRSLHYQFLSWYHWTLP 383
            R    + S L  P ++           I   L  SNFIG+ FSRSLHYQF  WY  TLP
Sbjct: 310 HRTGESILSLLRDPSKRKVPPQPLTPNQIVSTLFTSNFIGICFSRSLHYQFYVWYFHTLP 369

Query: 384 ILIF-----WSGMPFFVGPIWYVLHEWCWNSYPPNS 414
            L++     W      +  +  + E  WN+YP  S
Sbjct: 370 YLLWAMPARWLTHLLRLLVLGLI--ELSWNTYPSTS 403
```

Drosophila Vi

```
>gi|3024221|sp|Q24332|NT56_DROVI LETHAL(2)NEIGHBOUR OF TID PROTEIN (NOT58)
          Length = 526

 Score =  145 bits (366), Expect = 3e-34
 Identities = 103/273 (37%), Positives = 157/273 (56%), Gaps = 17/273 (6%)

Query: 33  VRYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQ 92
           ++Y+ F+  A  IV  L++L E+++  ++I++V YTEID+KAYM++ E   L+G  +YS
Sbjct: 34  IKYLAFEPAALPIVSVLIVLAEAVINVLVIQRVPYTEIDWKAYMQECEGF-LNGTTNYSL 92

Query: 93  VSGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACYYLLH-LP 151
           + G TGPLVYPA  V IY  +Y+LT    +V   Q  F  +YLL + L +  Y     +P
Sbjct: 93  LRGDTGPLVYPAAFVYIYSGLYYLTGQGTNVRLAQYIFACIYLLQMCLVLRLYTKSRKVP 152

Query: 152 PWCVVLACL-SKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKKSLALVIS 210
           P+ +VL+   S R+HSIYVLRLFND    L     +L A +     QR  L        S
Sbjct: 153 PYVLVLSAFTSYRIHSIYVLRLFNDPVAIL-----LLYAALNLFLDQRWTLG-------S 200

Query: 211 ATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVAVPFLRSFPQQ 270
             YS+AV +KMN  +   A  + LF L +  V+ TL+ L      Q+ +  PFLR+ P +
Sbjct: 201 ICYSLAVGVKMN--ILLFAPALLLFYLANLGVLRTLVQLTICAVLQLFIGAPFLRTHPME 258
```

FIG. 16-2

```
Query: 271 YLHCAFNFGRKFMYQWSINWQMMDEEAFNDKRF 303
           YL  +F+ GR F ++W++N++ + +E F  + F
Sbjct: 259 YLRGSFDLGRIFEHKWTVNYRFLSKELFEQREF 291

 Score = 53.3 bits (127), Expect = 1e-06
 Identities = 31/62 (50%), Positives = 41/62 (66%), Gaps = 6/62 (9%)

Query: 352 IPFVLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLH--EWCWNS 409
           +PF L   NFIGV  +RSLHYQF  WY  +LP L+ WS  P+ +G  + +L   E+CWN+
Sbjct: 412 LPFFL--CNFIGVACARSLHYQFYIWYFHSLPYLV-WS-TPYSLGVRYLILGIIEYCWNT 467

Query: 410 YP 411
           YP
Sbjct: 468 YP 469
```

Drosophila melanogaster

```
>gi|3024222|sp|Q27333|NT56_DROME LETHAL(2)NEIGHBOUR OF TID PROTEIN (NOT56)
(NOT45)
         Length = 510

 Score =  121 bits (305), Expect = 3e-27
 Identities = 96/272 (35%), Positives = 154/272 (56%), Gaps = 17/272 (6%)

Query: 34  RYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQV 93
            +Y++ +  A  IV   ++L E ++  ++I++V YTEID+ AYM++ E   L+G  +YS +
Sbjct: 36  KYLLLEPAALPIVGLFVLLAELVINVVVIQRVPYTEIDWVAYMQECEGF-LNGTTNYSLL 94

Query: 94  SGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACYYLLH-LPP 152
             G TGPLVYPA  V IY  +Y++T    +V   Q  F  +YLL LAL +  Y     +PP
Sbjct: 95  RGDTGPLVYPAAFVYIYSALYYVTSHGTNVRLAQYIFAGIYLLQLALVLRLYSKSRKVPP 154

Query: 153 WCVVLACL-SKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKKSLALVISA 211
           + +VL+  S R+HSIYVLRLFND      + V +L A +     +R  L        S
Sbjct: 155 YVLVLSAFTSYRIHSIYVLRLFNDP-----VAVLLLYAALNLFLDRRWTLG-------ST 202

Query: 212 TYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVAVPFLRSFPQQY 271
            +S+AV +KMN  +   A  + LF L +  ++ T+L L      Q+ +   PFL + P +Y
Sbjct: 203 FFSLAVGVKMN--ILLFAPALLLFYLANLGLLRTILQLAVCGVIQLLLGAPFLLTHPVEY 260

Query: 272 LHCAFNFGRKFMYQWSINWQMMDEEAFNDKRF 303
            L  +F+ GR F ++W++N++ +  + F ++ F
Sbjct: 261 LRGSFDLGRIFEHKWTVNYRFLSRDVFENRTF 292

 Score = 49.4 bits (117), Expect = 2e-05
 Identities = 27/60 (45%), Positives = 35/60 (58%), Gaps = 2/60 (3%)

Query: 352 IPFVLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYP 411
           +PF L   N +GV  SRSLHYQF  WY  +LP L + +      V  +   L E+CWN+YP
Sbjct: 407 LPFFL--CNLVGVACSRSLHYQFYVWYFHSLPYLAWSTPYSLGVRCLILGLIEYCWNTYP 464
```

FIG. 16-3

```
Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 28883317
Number of Sequences: 96469
Number of extensions: 1107545
Number of successful extensions: 2870
Number of sequences better than 10.0: 16
Number of HSP's better than 10.0 without gapping: 5
Number of HSP's successfully gapped in prelim test: 11
Number of HSP's that attempted gapping in prelim test: 2839
Number of HSP's gapped (non-prelim): 23

length of query: 458
length of database: 35,174,128
effective HSP length: 45
effective length of query: 413
effective length of database: 30,833,023
effective search space: 12734038499
effective search space used: 12734038499
T: 11
A: 40
X1: 15 ( 7.1 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 40 (21.8 bits)
S2: 67 (30.4 bits)
```

FIG.16-4

S. cerevisiae ALG3
ATGGAAGGTGAACAGTCTCCGCAAGGTGAAAAGTCTCTGCAAAGGAAGC
AATTTGTCAGACCTCCGCTGGATCTGTGGCAGGATCTCAAGGACGGTGTG
CGCTACGTGATCTTCGATTGTAGGGCCAATCTTATCGTTATGCCCCTTTTG
ATTTTGTTCGAAAGCATGCTGTGCAAGATTATCATTAAGAAGGTAGCTTAC
ACAGAGATCGATTACAAGGCGTACATGGAGCAGATCGAGATGATTCAGCT
CGATGGCATGCTGGACTACTCTCAGGTGAGTGGTGGAACGGGCCCGCTGG
TGTATCCAGCAGGCCACGTCTTGATCTACAAGATGATGTACTGGCTAACA
GAGGGAATGGACCACGTTGAGCGCGGGCAAGTGTTTTTCAGATACTTGTA
TCTCCTTACACTGGCGTTACAAATGGCGTGTTACTACCTTTTACATCTACC
ACCGTGGTGTGTGGTCTTGGCGTGCCTCTCTAAAAGATTGCACTCTATTTA
CGTGCTACGGTTATTCAATGATTGCTTCACTACTTTGTTTATGGTCGTCACG
GTTTTGGGGGCTATCGTGGCCAGCAGGTGCCATCAGCGCCCCAAATTAAA
GAAGTCCCTTGCGCTGGTGATCTCCGCAACATACAGTATGGCTGTGAGCA
TTAAGATGAATGCGCTGTTGTATTTCCCTGCAATGATGATTTCTCTATTCAT
CCTTAATGACGCGAACGTAATCCTTACTTTGTTGGATCTCGTTGCGATGAT
TGCATGGCAAGTCGCAGTTGCAGTGCCCTTCCTGCGCAGCTTTCCGCAACA
GTACCTGCATTGCGCTTTTAATTTCGGCAGGAAGTTTATGTACCAATGGAG
TATCAATTGGCAAATGATGGATGAAGAGGCTTTCAATGATAAGAGGTTCC
ACTTGGCCCTTTTAATCAGCCACCTGATAGCGCTCACCACACTGTTCGTCA
CAAGATACCCTCGCATCCTGCCCGATTTATGGTCTTCCCTGTGCCATCCGC
TGAGGAAAAATGCAGTGCTCAATGCCAATCCCGCCAAGACTATTCCATTC
GTTCTAATCGCATCCAACTTCATCGGCGTCCTATTTTCAAGGTCCCTCCAC
TACCAGTTTCTATCCTGGTATCACTGGACTTTGCCTATACTGATCTTTTGGT
CGGGAATGCCCTTCTTCGTTGGTCCCATTTGGTACGTCTTGCACGAGTGGT
GCTGGAATTCCTATCCACCAAACTCACAAGCAAGCACGCTATTGTTGGCA
TTGAATACTGTTCTGTTGCTTCTATTGGCCTTGACGCAGCTATCTGGTTCGG
TCGCCCTCGCCAAAAGCCATCTTCGTACCACCAGCTCTATGGAAAAAAAG
CTCAACTGA

S. cerevisiae Alg3p
MEGEQSPQGEKSLQRKQFVRPPLDLWQDLKDGVRYVIFDCRANLIVMPLLIL
FESMLCKIIIKKVAYTEIDYKAYMEQIEMIQLDGMLDYSQVSGGTGPLVYPAG
HVLIYKMMYWLTEGMDHVERGQVFFRYLYLLTLALQMACYYLLHLPPWCV
VLACLSKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVASRCHQRPKLKKSLALV
ISATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLVAMIAWQVAVA
VPFLRSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFNDKRFHLALLISHL
IALTTLFVTRYPRILPDLWSSLCHPLRKNAVLNANPAKTIPFVLIASNFIGVLFS
RSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWNSYPPNSQASTL
LLALNTVLLLLLALTQLSGSVALAKSHLRTTSSMEKKLN

FIG. 17

P. pastoris ALG3

ATGCCTCCGATAGAGCCAGCTGAAAGGCCAAAGCTTACGCTGAAAAATGT
TATCGGTGATCTAGTGGCTCTTATTCAAAACGTTTTATTTAACCCAGATTTT
AGTGTCTTCGTTGCACCTCTTTTATGGTTAGCTGATTCCATTGTTATCAAGG
TGATCATTGGCACTGTTTCCTACACAGATATTGATTTTTCTTCATATATGCA
ACAAATCTTTAAAATTCGACAAGGAGAATTAGATTATAGCAACATATTTG
GTGACACCGGTCCATTGGTTTACCCAGCCGGCCATGTTCATGCTTACTCAG
TACTTTCGTGGTACAGTGATGGTGGAGAAGACGTCAGTTTCGTTCAACAA
GCATTTGGTTGGTTATACCTAGGTTGCTTGTTACTATCCATCAGCTCCTACT
TTTTCTCTGGCTTAGGGAAAATACCTCCGGTTTATTTTGTTTTGTTGGTAGC
GTCCAAGAGACTGCATTCAATATTTGTATTGAGACTCTTCAATGACTGTTT
AACAACATTTTTGATGTTGGCAACTATAATCATCCTTCAACAAGCAAGTAG
CTGGAGGAAAGATGGCACAACTATTCCATTATCTGTCCCTGATGCTGCAG
ATACGTACAGTTTAGCCATCTCTGTAAAGATGAATGCGCTGCTATACCTCC
CAGCATTCCTACTACTCATATATCTCATTTGTGACGAAAATTTGATTAAAG
CCTTGGCACCTGTTCTAGTTTTGATATTGGTGCAAGTAGGAGTCGGTTATT
CGTTCATTTTACCGTTGCACTATGATGATCAGGCAAATGAAATTCGTTCTG
CCTACTTTAGACAGGCTTTTGACTTTAGTCGCCAATTTCTTTATAAGTGGA
CGGTTAATTGGCGCTTTTTGAGCCAAGAAACTTTCAACAATGTCCATTTTC
ACCAGCTCCTGTTTGCTCTCCATATTATTACGTTAGTCTTGTTCATCCTCAA
GTTCCTCTCTCCTAAAAACATTGGAAAACCGCTTGGTAGATTTGTGTTGGA
CATTTTCAAATTTTGGAAGCCAACCTTATCTCCAACCAATATTATCAACGA
CCCAGAAAGAAGCCCAGATTTTGTTTACACCGTCATGGCTACTACCAACTT
AATAGGGGTGCTTTTTGCAAGATCTTTACACTACCAGTTCCTAAGCTGGTA
TGCGTTCTCTTTGCCATATCTCCTTTACAAGGCTCGTCTGAACTTTATAGCA
TCTATTATTGTTTATGCCGCTCACGAGTATTGCTGGTTGGTTTTCCCAGCTA
CAGAACAAAGTTCCGCGTTGTTGGTATCTATCTTACTACTTATCCTGATTC
TCATTTTTACCAACGAACAGTTATTTCCTTCTCAATCGGTCCCTGCAGAAA
AAAAGAATACATAA

P. pastoris Alg3p

MPPIEPAERPKLTLKNVIGDLVALIQNVLFNPDFSVFVAPLLWLADSIVIKVIIG
TVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYPAGHVHAYSVLSWYS
DGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSIF
VLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVKMN
ALLYLPAFLLLIYLICDENLIKALAPVLVLILVQVGVGYSFILPLHYDDQANEIR
SAYFRQAFDFSRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFILKF
LSPKNIGKPLGRFVLDIFKFWKPTLSPTNIINDPERSPDFVYTVMATTNLIGVLF
ARSLHYQFLSWYAFSLPYLLYKARLNFIASIIVYAAHEYCWLVFPATEQSSAL
LVSILLLILILIFTNEQLFPSQSVPAEKKNT

FIG. 18

P. pastoris ALG3 BLAST

```
Sequences producing significant alignments:                      (bits) Value

gi|586444|sp|P38179|ALG3_YEAST  Dolichyl-P-Man:Man(5)GlcNAc(...228    2e-58
gi|12802365|gb|AAK07848.1|AF309689_10  putative NOT-56 manno...212    8e-54
gi|984725|gb|AAA75352.1|  ORF 1                                206    4e-52
gi|7492702|pir||T39084  probable mannosyltransferase - fissi...176    8e-43
gi|16226531|gb|AAL16193.1|AF428424_1  At2g47760/F17A22.15 [A...164    2e-39
gi|25367230|pir||B84919  Not56-like protein [imported] - Ara...164    3e-39
gi|25814791|emb|CAB70171.2|  Hypothetical protein K09E4.2 [C...161    2e-38
gi|17535001|ref|NP_496950.1|  Putative plasma membrane membr...160    3e-38
gi|1654000|emb|CAA70220.1|  Not56-like protein [Homo sapiens...155    2e-36
gi|13279206|gb|AAH04313.1|AAH04313  Unknown (protein for IMA...154    2e-36
gi|22122365|ref|NP_666051.1|  hypothetical protein MGC36684 ...150    3e-35
gi|21292031|gb|EAA04176.1|  agCP3388 [Anopheles gambiae str....120    4e-26
gi|1780792|emb|CAA71167.1|  lethal(2)neighbour of tid [Droso...114    3e-24
```

Alignments

S. cerevisiae

```
 Score =  228 bits (580), Expect = 2e-58
 Identities = 154/429 (35%), Positives = 229/429 (53%), Gaps = 37/429 (8%)

Query: 9   RPKLTLKNVIGDLVALIQNVLFNPDFSVFVAPLLWLADSIVIKVIIGTVSYTDIDFSSYM 68
           RP L L     DL   ++ V+F+    ++V PLL L +S++ K+II  V+YT+ID+ +YM
Sbjct: 20  RPPLDLWQ---DLKDGVRYVIFDCRANLIVMPLLILFESMLCKIIIKKVAYTEIDYKAYM 76

Query: 69  QQIFKIR-QGELDYSNIFGDTGPLVYPAGHVHAYSVLSWYSDGGEDVSFVQQAFGWLYLG 127
           +QI  I+  G LDYS + G TGPLVYPAGHV  Y ++ W ++G + V   Q  F +LYL
Sbjct: 77  EQIEMIQLDGMLDYSQVSGGTGPLVYPAGHVLIYKMMYWLTEGMDHVERGQVFFRYLYLL 136

Query: 128 CLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSIFVLRLFNDCLTTFLMLATI---IILQ 184
            L L++  Y+   L  +PP   VL   SKRLHSI+VLRLFNDC TT  M+ T+   I+
Sbjct: 137 TLALQMACYY---LLHLPPWCVVLACLSKRLHSIYVLRLFNDCFTTLFMVVTVLGAIVAS 193

Query: 185 QASSWRKDGTTIPLSVPDAADTYSLAISVKMNXXXXXXXXXXXXXXXCDENLIKALAPXX 244
           +     K   ++ L +   + TYS+A+S+KMN                D N+I  L
Sbjct: 194 RCHQRPKLKKSLALVI---SATYSMAVSIKMNALLYFPAMMISLFILNDANVILTLLDLV 250

Query: 245 XXXXXXXXXXYSFILPLHYDDQANEIRSAYFRQAFDFSRQFLYKWTVNWRFLSQETFNNV 304
                       F+               Y   AF+F R+F+Y+W++NW+ + +E FN+
Sbjct: 251 AMIAWQVAVAVPFL---------RSFPQQYLHCAFNFGRKFMYQWSINWQMMDEEAFNDK 301

Query: 305 HFHQLLFALHIITL-VLFILKFLSPKNIGKPLGRFVLDIFKFWKPTLSPTNIIN-DPERS 362
            FH  L   H+I L  LF+ ++           R + D++      L    ++N +P ++
Sbjct: 302 RFHLALLISHLIALTTLFVTRY----------PRILPDLWSSLCHPLRKNAVLNANPAKT 351

Query: 363 PDFVYTVMATTNLIGVLFARSLHYQFLSWYAFSLPYLLYKARLNFIASIIVYAAHEYCWL 422
             F   V+  +N IGVLF+RSLHYQFLSWY ++LP L++ + + F    I Y  HE+CW
Sbjct: 352 IPF---VLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWSGMPFFVGPIWYVLHEWCWN 408

Query: 423 VFPATEQSS 431
            +P   Q+S
Sbjct: 409 SYPPNSQAS 417
```

FIG. 19-1

Neurospora crassa

```
Score =  212 bits (540), Expect = 8e-54
Identities = 140/400 (35%), Positives = 212/400 (53%), Gaps = 29/400 (7%)

Query: 35   SVFVAPLLWLADSIVIKVIIGTVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYP 94
            S  + P L+L D+++  +II  V YT+ID+++YM+Q+ +I  GE DY+ + G TGPLVYP
Sbjct: 33   SKLIPPALFLVDALLCGLIIWKVPYTEIDWAAYMEQVSQILSGERDYTKVRGGTGPLVYP 92

Query: 95   AGHVHAYSVLSWYSDGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVA 154
            A HV+ Y+ L   +D G ++   QQ F  LY+  L + +  Y+       K PP  F LL
Sbjct: 93   AAHVYIYTGLYHLTDEGRNILLAQQLFAGLYMVTLAVVMGCYW---QAKAPPYLFPLLTL 149

Query: 155  SKRLHSIFVLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVK 214
            SKRLHSIFVLR FNDC      +    I    Q+  +W+             A  Y+L + VK
Sbjct: 150  SKRLHSIFVLRCFNDCFAVLFLWLAIFFFQR-RNWQA-----------GALLYTLGLGVK 197

Query: 215  MNXXXXXXXXXXXXXXXXCDENLIKALAPXXXXXXXXXXXXXYSFILPLHYDDQANEIRSAY 274
            M               + +  L                    F+    HY  +         Y
Sbjct: 198  MTLLLSLPAVGIVLFLGSG-SFVTTLQLVATMGLVQILIGVPFL--AHYPTE-------Y 247

Query: 275  FRQAFDFSRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFI-LKFLSPKNIGK 333
              +AF+  SRQF +KWTVNWRF+ +E F +   F   L ALH++ L +FI  +++ P    K
Sbjct: 248  LSRAFELSRQFFFKWTVNWRFVGEEIFLSKGFALTLLALHVLVLGIFITTRWIKPAR--K 305

Query: 334  PLGRFVLDIFKFWKPTLS-PTNIINDPERSPDFVYTVMATTNLIGVLFARSLHYQFLSWY 392
              L + +  +     KP L+ P +      + +P ++ T + + N +G+LFARSLHYQF ++
Sbjct: 306  SLVQLISPVLLAGKPPLTVPEHRAAARDVTPRYIMTTILSANAVGLLFARSLHYQFYAYV 365

Query: 393  AFSLPYLLYKARLNFIASIIVYAAHEYCWLVFPATEQSSA 432
            A+S P+LL++A L+  +   +++A HE+ W VFP+T  SSA
Sbjct: 366  AWSTPFLLWRAGLHPVLVYLLWAVHEWAWNVFPSTPASSA 405
```

Schizosaccharomyces pombe

```
Score =  176 bits (445), Expect = 8e-43
Identities = 132/390 (33%), Positives = 194/390 (49%), Gaps = 35/390 (8%)

Query: 42   LWLADSIVIKVIIGTVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYPAGHVHAY 101
            L L +   +  II  V YT+ID+ +YM+Q+        GE DY  ++ G TGPLVYP GHV  Y
Sbjct: 30   LLLLEIPFVFAIISKVPYTEIDWIAYMEQVNSFLLGERDYKSLVGCTGPLVYPGGHVFLY 89

Query: 102  SVLSWYSDGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSI 161
            ++L + +DGG ++   Q  F ++Y   +  +I  Y F  + +  P   +VLL+ SKRLHSI
Sbjct: 90   TLLYYLTDGGTNIVRAQYIFAFVYW--ITTAIVGYLFK-IVRAPFYIYVLLILSKRLHSI 146

Query: 162  FVLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVKMNXXXXX 221
            F+LRLFND   +L  + I+       W +                A+   S+A SVKM+
Sbjct: 147  FILRLFNDGFNS-LFSSLFILSSCKKWVR-----------ASILLSVACSVKMSSLLYV 194

Query: 222  XXXXXXXXXXCDENLIKALAPXXXXXXXXXXXXXYSFILPLHYDDQANEIRSAYFRQAFDF 281
                             L++ L P                  +  +  +            +Y+ QAFDF
Sbjct: 195  PAYLVL--------LLQILGPKKTWMHIFVIIIVQILFSIPF----LAYFWSYWTQAFDF 242

Query: 282  SRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFILKFLSPKNIGKPLGRFVLD 341
            R F YKWTVNWRF+ +   F +  F     +  LH+  LV F  K  +  +     P
Sbjct: 243  GRAFDYKWTVNWRFIPRSIFESTSFSTSILFLHVALLVAFTCKHWNKLSRATP------- 295

Query: 342  IFKFWKPTLSPTNIINDPERSPDFVYTVMATTNLIGVLFARSLHYQFLSWYAFSLPYLLY 401
             F       L+    +        +P+F++T +AT+NLIG+L ARSLHYQF +W+A+   PYL Y
```

FIG. 19-2

```
Sbjct: 296 -FAMVNSMLTLKPLPKLQLATPNFIFTALATSNLIGILCARSLHYQFYAWFAWYSPYLCY 354

Query: 402 KARLNFIASIIVYAAHEYCWLVFPATEQSS 431
           +A        I ++    EY W VFP+T+ SS
Sbjct: 355 QASFPAPIVIGLWMLQEYAWNVFPSTKLSS 384
Arabidopsis thaliana

 Score =  164 bits (415), Expect = 2e-39
 Identities = 131/391 (33%), Positives = 194/391 (49%), Gaps = 29/391 (7%)

Query: 42   LWLADSIVIKVIIGTVSYTDIDFSSYMQQIFKIRQGELDYSNIFGDTGPLVYPAGHVHAY 101
             L LAD+I++ +II  V YT ID+ +YM Q+     GE DY N+ GDTGPLVYPAG ++ Y
Sbjct: 39   LILADAILVALIIAYVPYTKIDWDAYMSQVSGFLGGERDYGNLKGDTGPLVYPAGFLYVY 98

Query: 102  SVLSWYSDGGEDVSFVQQAFGWLYLGCLLLSISSYFFSGLGKIPPVYFVLLVASKRLHSI 161
             S +    + G  +V    Q  FG LY+  L + +  Y  + +  +P     LL  SKR+HSI
Sbjct: 99   SAVQNLTGG--EVYPAQILFGVLYIVNLGIVLIIYVKTDV--VPWWALSLLCLSKRIHSI 154

Query: 162  FVLRLFNDCLTTFLMLATIIILQQASSWRKDGTTIPLSVPDAADTYSLAISVKMNXXXXX 221
             FVLRLFNDC     L+ A++ +       +RK      + +              +S A+SVKMN
Sbjct: 155  FVLRLFNDCFAMTLLHASMALFL----YRKWHLGMLV--------FSGAVSVKMNVLLYA 202

Query: 222  XXXXXXXXXXCDENLIKALAPXXXXXXXXXXXXXYSFILPLHYDDQANEIRSAYFRQAFDF 281
                        N+I  ++                F++               +Y   AFD
Sbjct: 203  PTLLLLLLKAM--NIIGVVSALAGAALAQILVGLPFLITYPV---------SYIANAFDL 251

Query: 282  SRQFLYKWTVNWRFLSQETFNNVHFHQLLFALHIITLVLFILKFLSPKNIGKPLGRFVLD 341
               R F++ W+VN++F+ +   F +  F    L    H+  LV F     +   K+ G  +G
Sbjct: 252  GRVFIHFWSVNFKFVPERVFVSKEFAVCLLIAHLFLLVAFA-NYKWCKHEGGIIGFMRSR 310

Query: 342  IFKFWKP-TLSPTNIINDPERSPDFVYTVMATTNLIGVLFARSLHYQFLSWYAFSLPYLL 400
              F     P +LS +++         + + V T M     N IG++FARSLHYQF SWY +SLPYLL
Sbjct: 311  HFFLTLPSSLSFSDVSASRIITKEHVVTAMFVGNFIGIVFARSLHYQFYSWYFYSLPYLL 370

Query: 401  YKARLNFIASIIVYAAHEYCWLVFPATEQSS 431
             ++       +I++     E CW V+P+T  SS
Sbjct: 371  WRTPFPFTWLRLIMFLGIELCWNVYPSTPSSS 401
```

FIG. 19-3

K. lactis ALG3

TTTGTTTACAAGCTGATACCAACGAACATGAATACACCGGCAGGTTTACT
GAAGATTGGCAAAGCTAACCTTTTACATCCTTTTACCGATGCTGTATTCAG
TGCGATGAGAGTAAACGCAGAACAAATTGCATACATTTTACTTGTTACCA
ATTACATTGGAGTACTATTTGCTCGATCATTACACTACCAATTCCTATCTT
GGTACCATTGGACGTTACCAGTACTATTGAATTGGGCCAATGTTCCGTATC
CGCTATGTGTGCTATGGTACCTAACACATGAGTGGTGCTGGAACAGCTAT
CCGCCAAACGCTACTGCATCCACACTGCTACACGCGTGTAACACATACTG
TTATTGGCTGTATTCTTAAGAGGACCCGCAAACTCGAAAAGTGGTGATAA
CGAAACAACACACGAGAAAGCTGAG

K. lactis Alg3p

FVYKLIPTNMNTPAGLLKIGKANLLHPFTDAVFSAMRVNAEQIAYILLVTNYI
GVLFARSLHYQFLSWYHWTLPVLLNWANVPYPLCVLWYLTHEWCWNSYPP
NATASTLLHACNTYCYWLYSZEDPQTRKVVITKQHTRKL

FIG. 20

K. lactis ALG3 BLAST

```
                                                                    Score     E
Sequences producing significant alignments:                         (bits)  Value

gi|586444|sp|P38179|ALG3_YEAST  Dolichyl-P-Man:Man(5)GlcNAc(...     125    1e-28
gi|984725|gb|AAA75352.1|  ORF 1                                      94    4e-19
gi|16226531|gb|AAL16193.1|AF428424_1  At2g47760/F17A22.15 [A...      72    1e-12
gi|25367230|pir||B84919  Not56-like protein [imported] - Ara...      72    1e-12
gi|21292031|gb|EAA04176.1|  agCP3388 [Anopheles gambiae str....      69    2e-11
gi|20892051|ref|XP_148657.1|  similar to Lethal(2)neighbour ...      65    2e-10
```

Alignments

S. cerevisiae

```
Score =  125 bits (314), Expect = 1e-28
Identities = 60/120 (50%), Positives = 83/120 (69%), Gaps = 1/120 (0%)
Frame = +3

Query: 66  ANLLHPFT-DAVFSAMRVNAEQIAYILLVTNYIGVLFARSLHYQFLSWYHWTLPVLLNWA 242
            ++L HP   +AV +A     A+ I ++L+ +N+IGVLF+RSLHYQFLSWYHWTLP+L+ W+
Sbjct: 332 SSLCHPLRKNAVLNANP--AKTIPFVLIASNFIGVLFSRSLHYQFLSWYHWTLPILIFWS 389

Query: 243 NVPYPLCVLWYLTHEWCWNSYPPNATASTLLHACNTYCYWLYS*EDPQTRKVVITKQHTR 422
             +P+ +   +WY+ HEWCWNSYPPN+ ASTLL A NT    L +     + V + K H R
Sbjct: 390 GMPFFVGPIWYVLHEWCWNSYPPNSQASTLLLALNTVLLLLLA-LTQLSGSVALAKSHLR 448
```

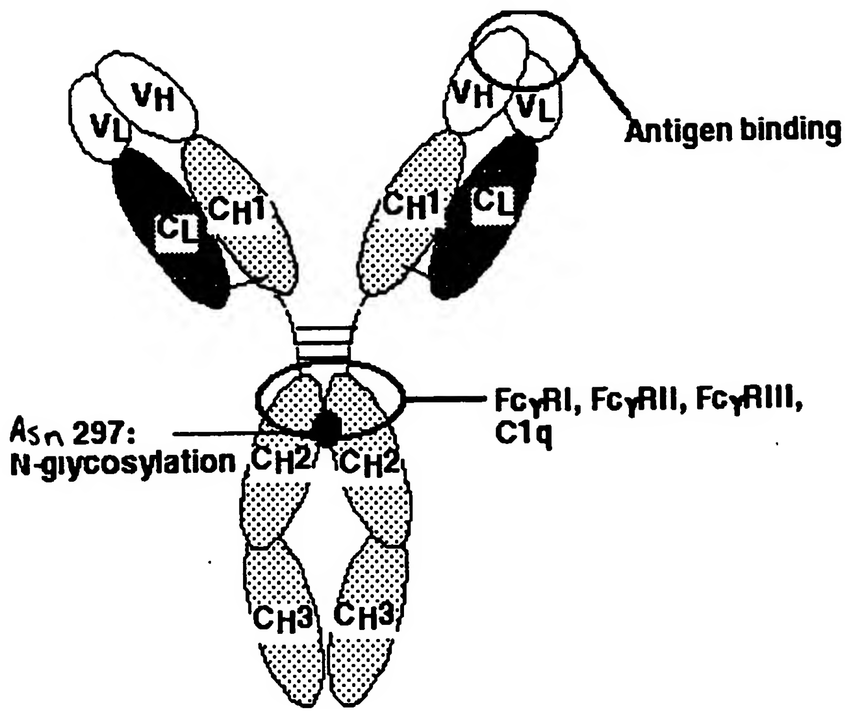
A. thaliana

```
Score = 72.0 bits (175), Expect = 1e-12
Identities = 42/107 (39%), Positives = 57/107 (53%), Gaps = 3/107 (2%)
Frame = +3

Query: 84  FTDAVFSAMRVNAEQIAYILLVTNYIGVLFARSLHYQFLSWYHWTLPVLLNWANVPYPLC 263
            F+D   S + +  E +    + V N+IG++FARSLHYQF SWY ++LP LL      P  L
Sbjct: 322 FSDVSASRI-ITKEHVVTAMFVGNFIGIVFARSLHYQFYSWYFYSLPYLLWRTPFPTWLR 380

Query: 264 VLWYLTHEWCWNSYPPNATASTL---LHACNTYCYWLYS*EDPQTRK 395
            ++ +L  E CWN YP   ++S L   LH         WL    DP    K
Sbjct: 381 LIMFLGIELCWNVYPSTPSSSGLLLCLHLIILVGLWLAPSVDPYQLK 427
```

FIG. 21



VH
VL
CL
CH1
VH
VL
CH1
CL
Antigen binding
FcγRI, FcγRII, FcγRIII,
C1q
Asn 297:
N-glycosylation
CH2
CH2
CH3
CH3

FIG. 22

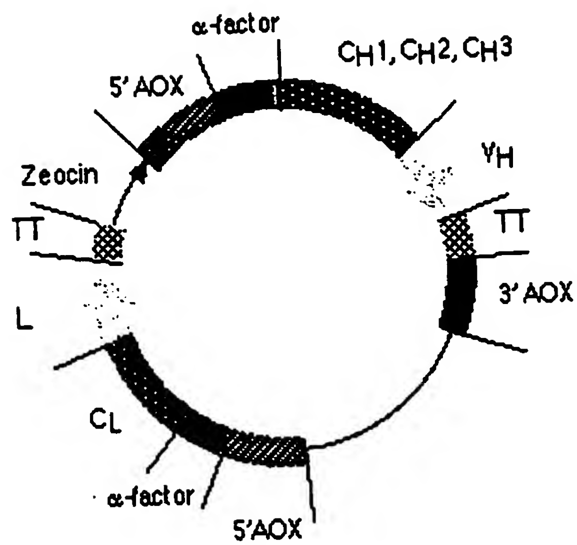

α-factor
CH1, CH2, CH3
5' AOX
VH
Zeocin
TT
TT
3' AOX
L
CL
α-factor
5'AOX

FIG. 23

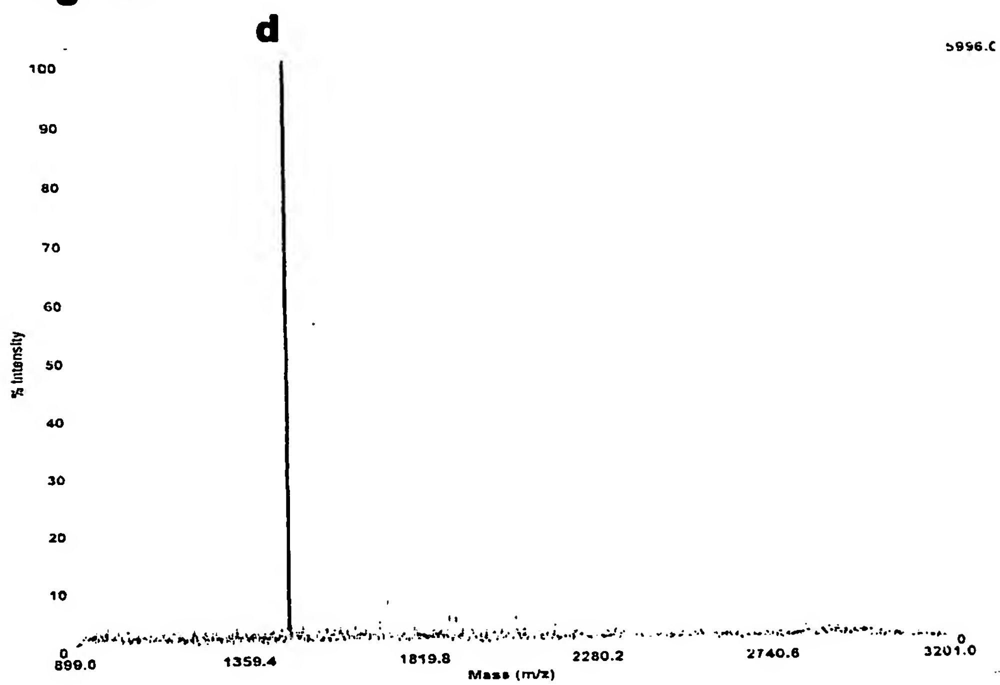
>gi|6754685|ref|NM_010795.1| Mus musculus mannoside acetyl
glucosaminyltransferase 3 (Mgat3), mRNA

ATGAAGATGAGACGCTACAAGCTCTTTCTCATGTTCTGTATGGCTGGCCTGTGCCTCATATCCTTCCTGC
ACTTCTTTAAGACCTTATCCTATGTCACCTTCCCGAGAGAACTGGCCTCCCTCAGCCCTAACCTCGTATC
CAGCTTCTTCTGGAACAATGCCCCTGTCACTCCCCAGGCCAGTCCGGAGCCGGGTGGCCCCGACCTATTG
CGGACACCCCTCTACTCCCACTCTCCCCTGCTCCAGCCACTGTCCCCGAGCAAGGCCACAGAGGAACTGC
ACCGGGTGGACTTCGTGTTGCCGGAGGACACCACGGAGTATTTTGTGCGCACCAAAGCTGGTGGTGTGTG
CTTCAAACCAGGTACCAGGATGCTGGAGAAACCTTCGCCAGGGCGGACAGAGGAGAAGCCCGAAGTGTCT
GAGGGCTCCTCAGCCCGGGGACCTGCTCGGAGGCCCATGAGGCACGTGTTGAGTACGCGGAGCGCCTGG
GCAGCCGGGGCACTAGGCGCAAGTGGGTTGAGTGTGTGTGCCTGCCAGGCTGGCACGGGCCCAGTTGCGG
GGTGCCCACGGTGGTGCAGTATTCCAACCTGCCCACCAAGGAACGCCTGGTACCCAGGGAGGTACCGAGG
CGGGTTATCAACGCCATCAACATCAACCACGAGTTCGACCTGCTGGATGTGCGCTTCCATGAGCTGGGAG
ATGTTGTGGACGCCTTCGTGGTCTGTGAATCTAATTTCACCGCCTACGGGGAGCCTCGGCCGCTCAAGTT
CCGAGAGATGCTGACCAATGGCACCTTCGAGTACATCCGCCACAAGGTGCTCTATGTCTTCCTGGACCAT
TTCCCACCTGGTGGCCGTCAGGACGGCTGGATTGCGGATGACTACCTGCGCACCTTCCTCACCCAGGATG
GCGTCTCCCGCCTGCGCAACCTGCGGCCCGATGACGTCTTTATCATCGACGATGCGGACGAGATCCCTGC
GCGTGATGGTGTGCTGTTCCTCAAACTCTACGATGGCTGGACAGAGCCCTTCGCCTTCCACATGCGGAAG
TCCCTGTATGGTTTCTTCTGGAAGCAGCCGGGCACACTGGAGGTGGTGTCAGGCTGCACCATGGACATGC
TGCAGGCCGTGTATGGGCTGGATGGCATCCGCCTGCGCCGCCGCCAGTACTACACCATGCCCAACTTCCG
GCAGTATGAGAACCGCACCGGCCACATCCTAGTGCAGTGGTCTCTCGGCAGCCCCCTGCACTTCGCGGGC
TGGCATTGCTCCTGGTGCTTCACACCCGAGGGCATCTACTTTAAACTCGTGTCAGCCCAGAATGGCGACT
TCCCCCGCTGGGGTGACTATGAGGACAAGAGGGACCTCAATTACATCCGCAGCTTGATCCGCACTGGGGG
ATGGTTCGACGGAACGCAGCAGGAGTACCCTCCTGCGGACCCCAGTGAGCACATGTATGCTCCTAAATAC
CTGCTCAAGAACTATGACCAGTTCCGCTACTTGCTGGAAAATCCCTACCGGGAGCCCAAGAGCACTGTAG
AGGGTGGGCGCCAGAACCAGGGCTCAGATGGAAGGCCATCTGCTGTCAGGGGCAAGTTGGATACAGTGGA
GGGCTAG

>gi|2117717|pir||JC4362 beta-1,4-mannosyl-glycoprotein 4-beta-N-
acetylglucosaminyltransferase (EC 2.4.1.144) III - mouse
MRRYKLFLMFCMAGLCLISFLHFFKTLSYVTFPRELASLSPNLISSFFWNNAPVTPQASPEPGDPDLLRT
PLYSHSPLLQPLSPSKATEELHRVDFVLPEDTTEYFVRTKAGGVCFKPGTRMLEKPSPGRTEEKTEVSEG
SSARGPARRPMRHVLSSRERLGSRGTRRKWVECVCLPGWHGPSCGVPTVVQYSNLPTKERLVPREVPRRV
INAININHEFDLLDVRFHELGDVVDAFVVCDSNFTAYGEPRPLKFREMLTNGTFEYIRHKVLYVFLDHFP
PGGRQDGWIADDYLRTFLTQDGVSRLRNLRPDDVFIIDDADEIPARDGVLFLKLYDGWTEPFAFHMRKSL
YGFFWKQPGTLEVVSGCTMDMLQAVYGLDGIRLRRRQYYTMPNFRQYENRTGHILVQWSLGSPLHFAGWH
CSWCFTPEGIYFKLVSAQNGDFPRWGDYEDKRDLNYIRSLIRTGGWFDGTQQEYPPADPSEHMYAPKYLL
KNYDQFRYLLENPYREPKSTVEGGRQNQGSDGRSSAVRGKLDTAEG

FIG. 24

Fig. 25


d
5996.0
100
90
80
70
60
50
40
30
20
10
0
% Intensity
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
Mass (m/z)

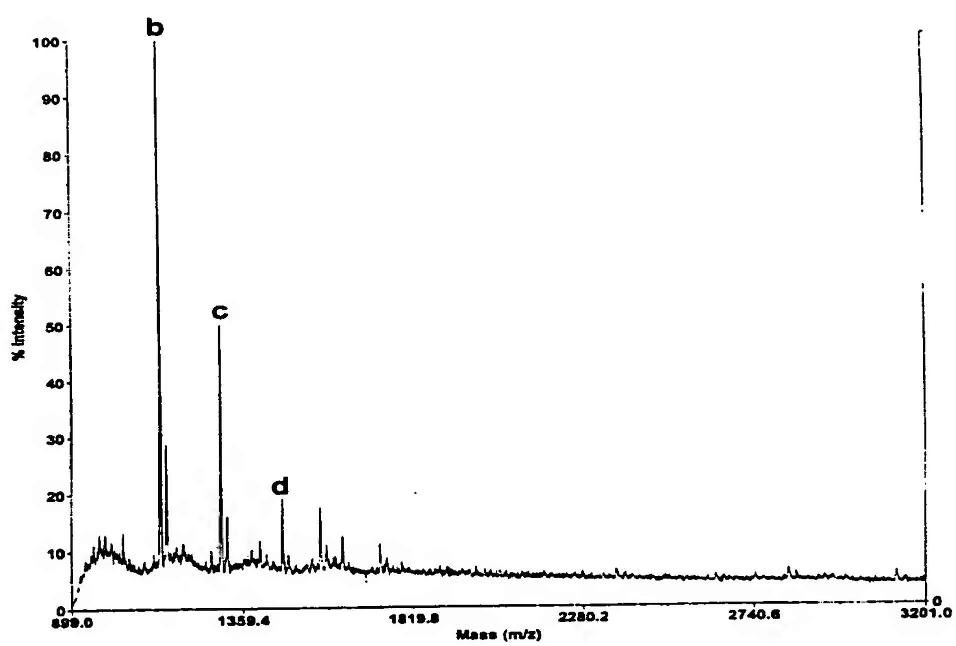
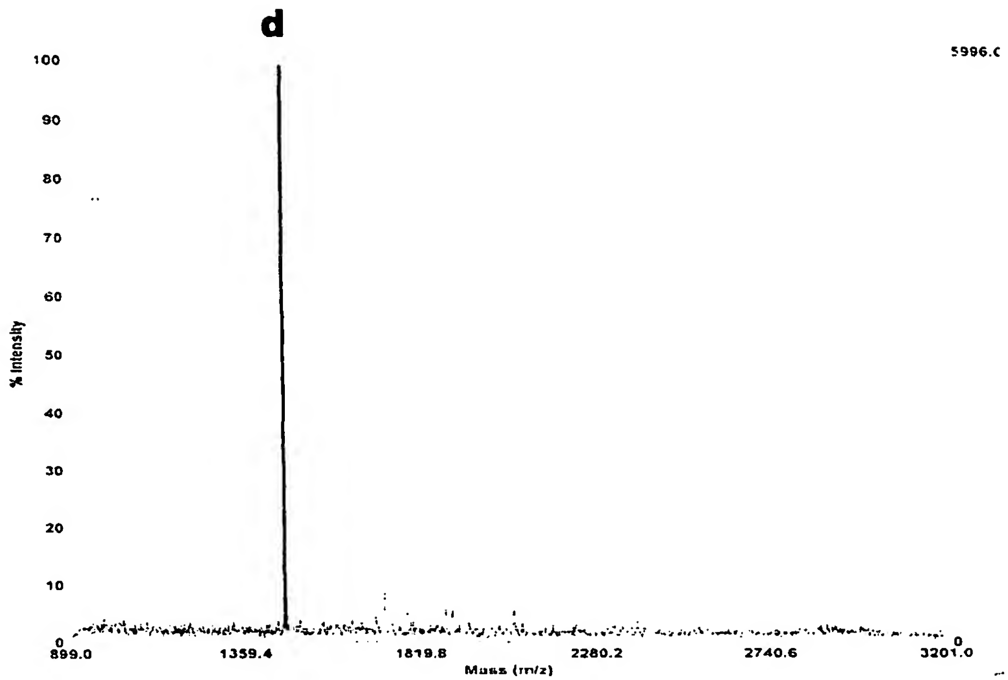

b
c
d
100
90
80
70
60
50
40
30
20
10
0
% Intensity
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
Mass (m/z)

Fig. 26


d
5996.0
100
90
80
70
60
50
40
30
20
10
0
% Intensity
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
Mass (m/z)

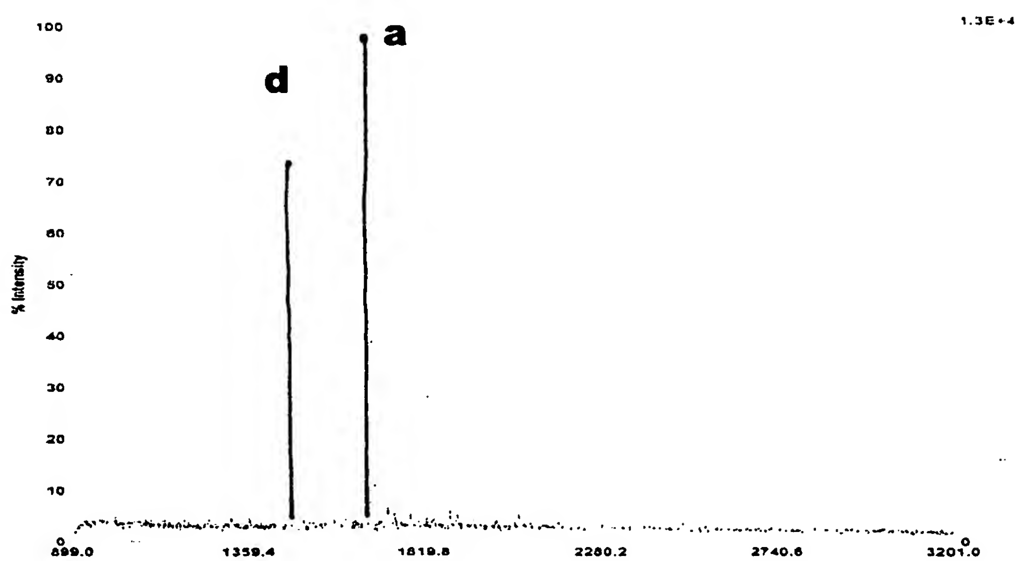

a
d
1.3E+4
100
90
80
70
60
50
40
30
20
10
0
% Intensity
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
Mass (m/z)

Fig. 27

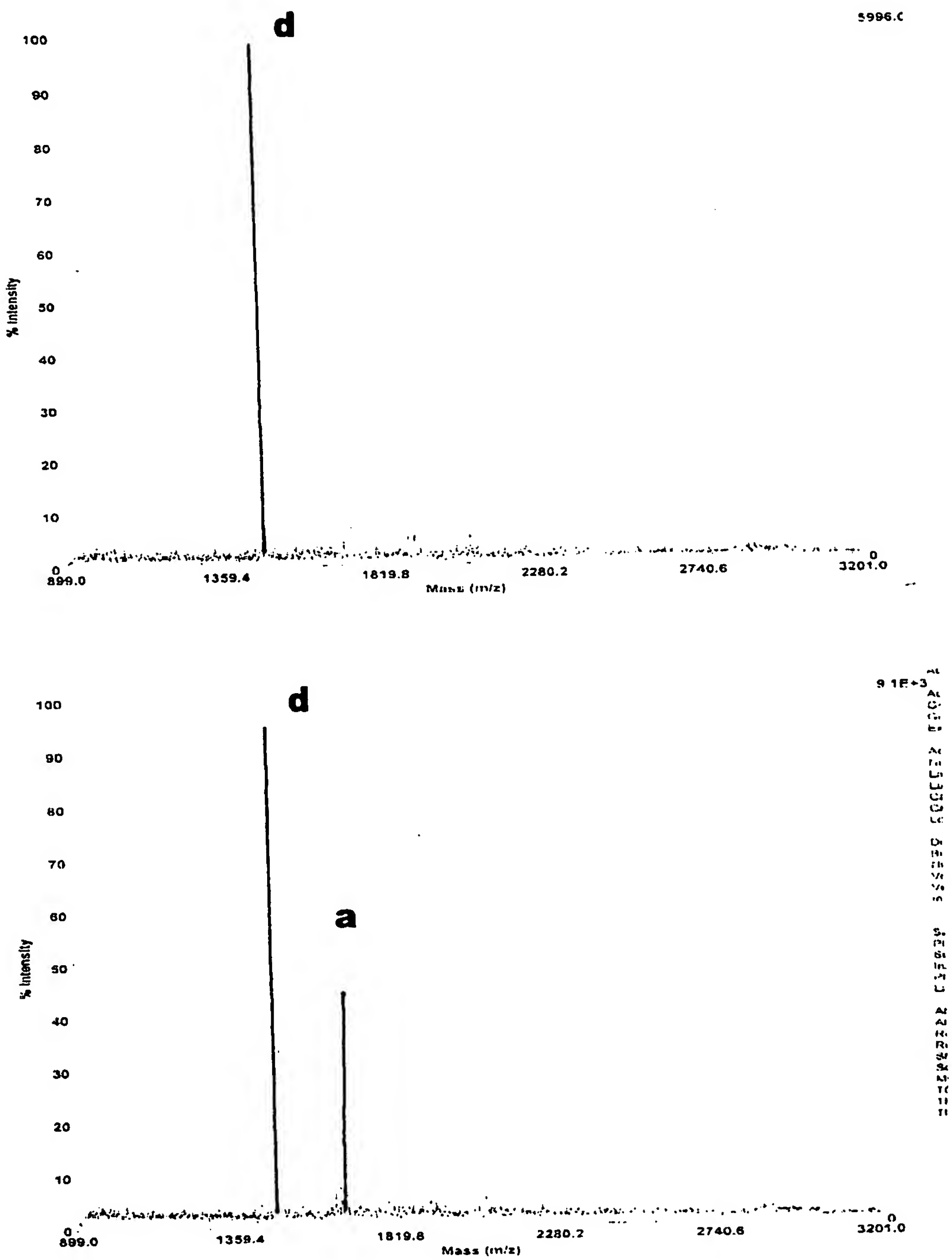
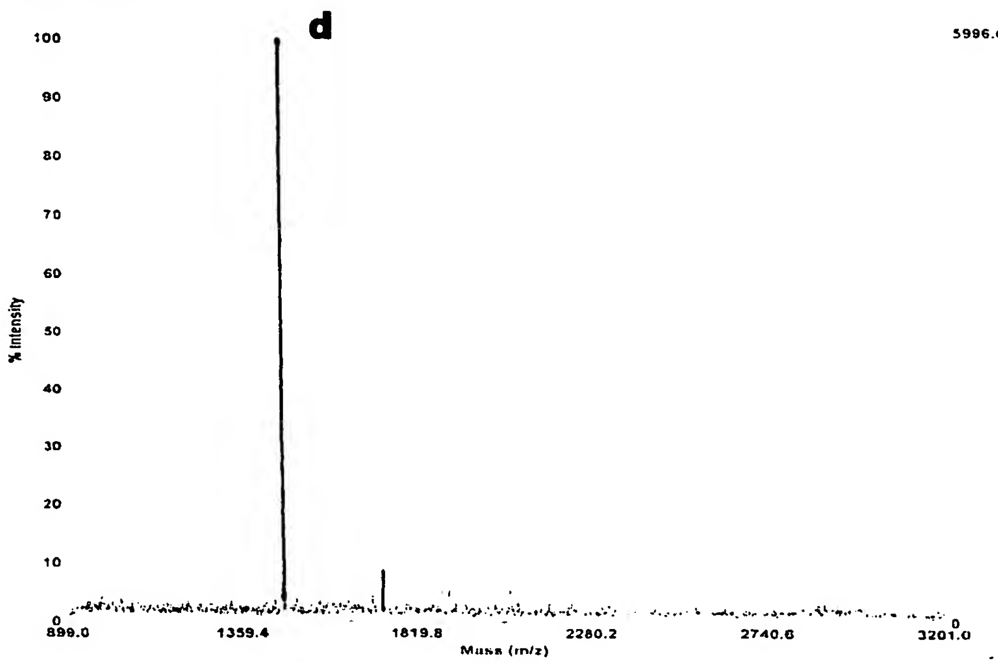

d
5996.0
% Intensity
Mass (m/z)
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
d
a
9.1E+3
% Intensity
Mass (m/z)

Fig. 28


d
5996.0
100
90
80
70
60
50
40
30
20
10
0
% Intensity
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
Mass (m/z)

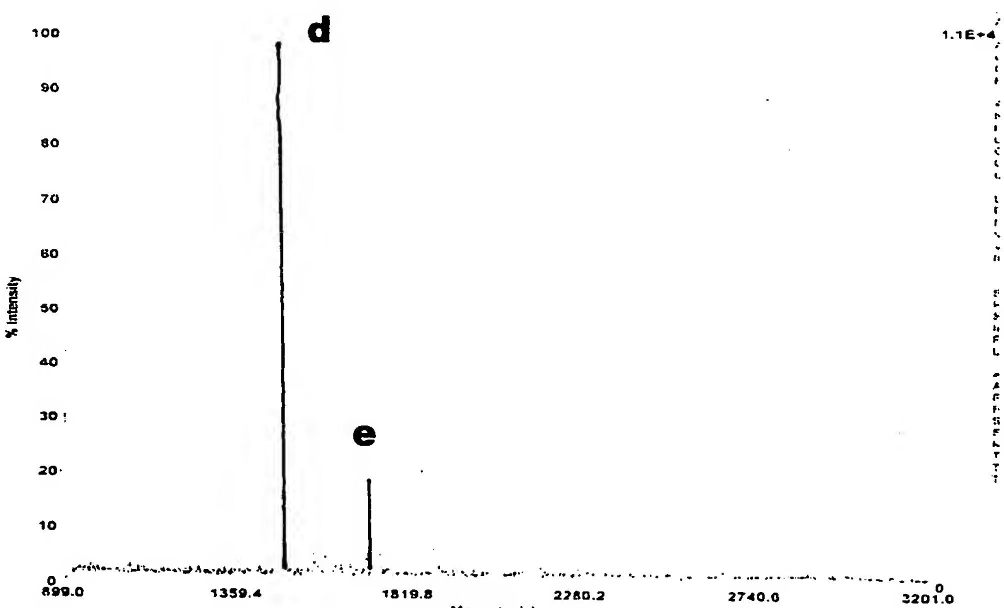

d
e
1.1E+4
100
90
80
70
60
50
40
30
20
10
0
% Intensity
899.0
1359.4
1819.8
2280.2
2740.6
3201.0
Mass (m/z)

Fig. 29

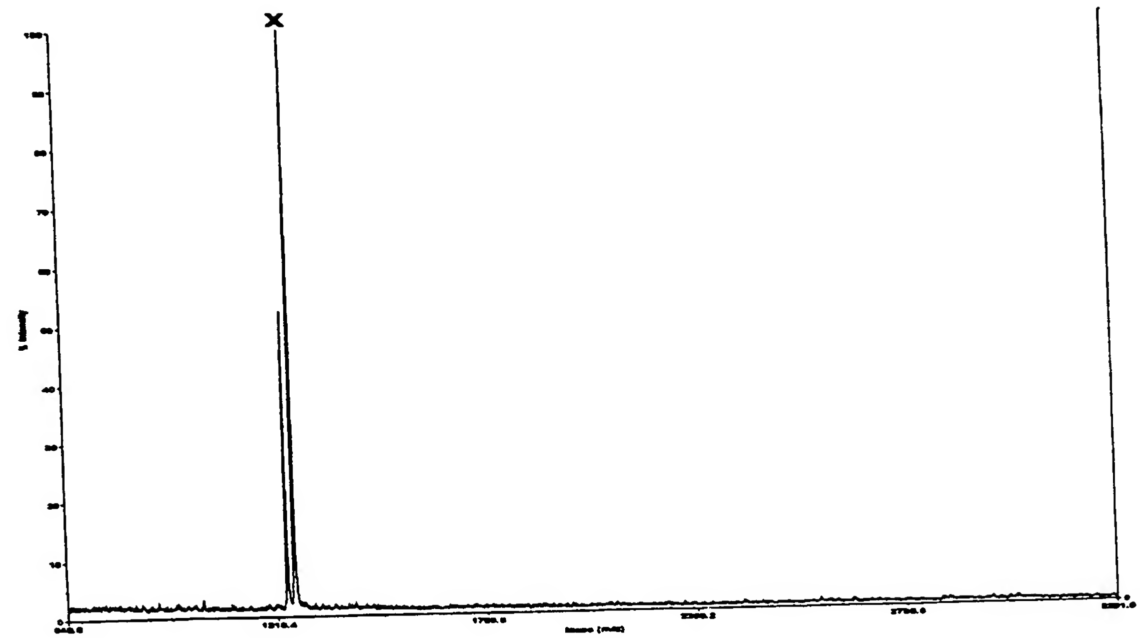

x

Fig. 30

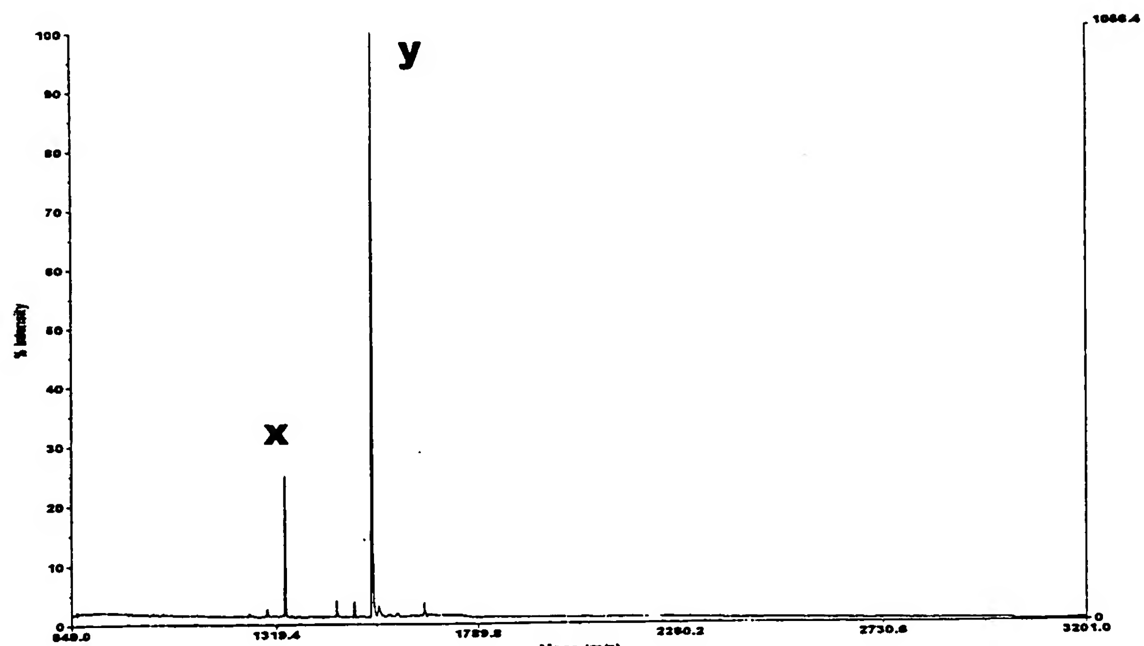

y
x
100
90
80
70
60
50
40
30
20
10
0
849.0
1319.4
1789.8
2260.2
2730.6
3201.0
Mass (m/z)
% Intensity

Fig. 31

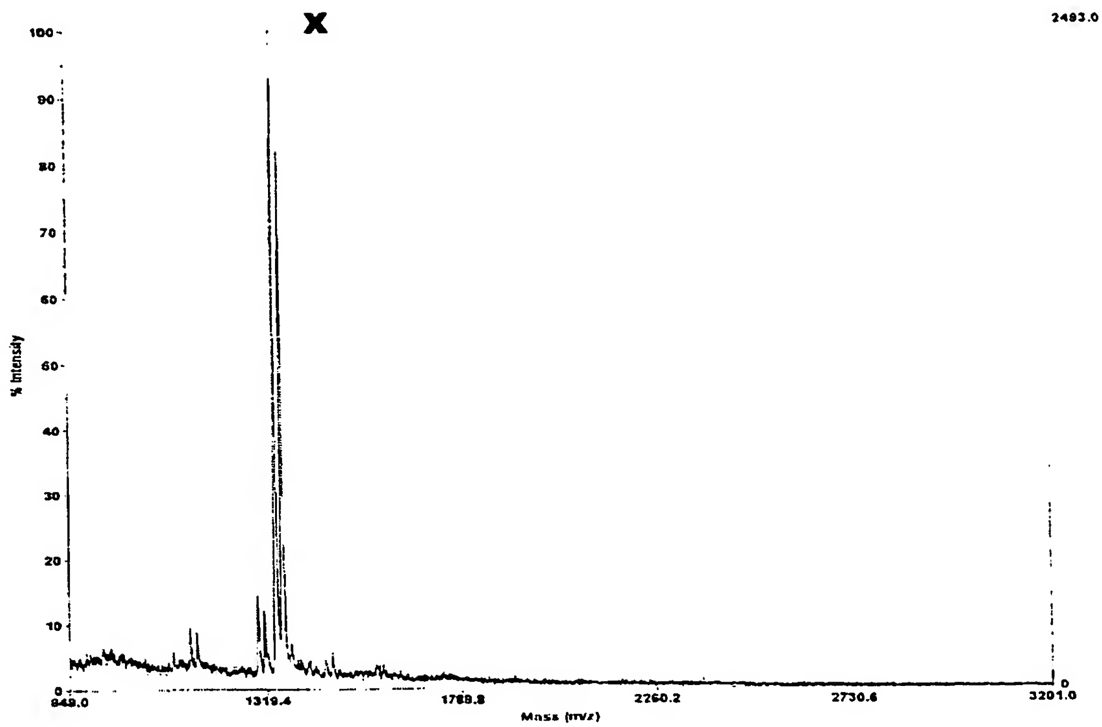
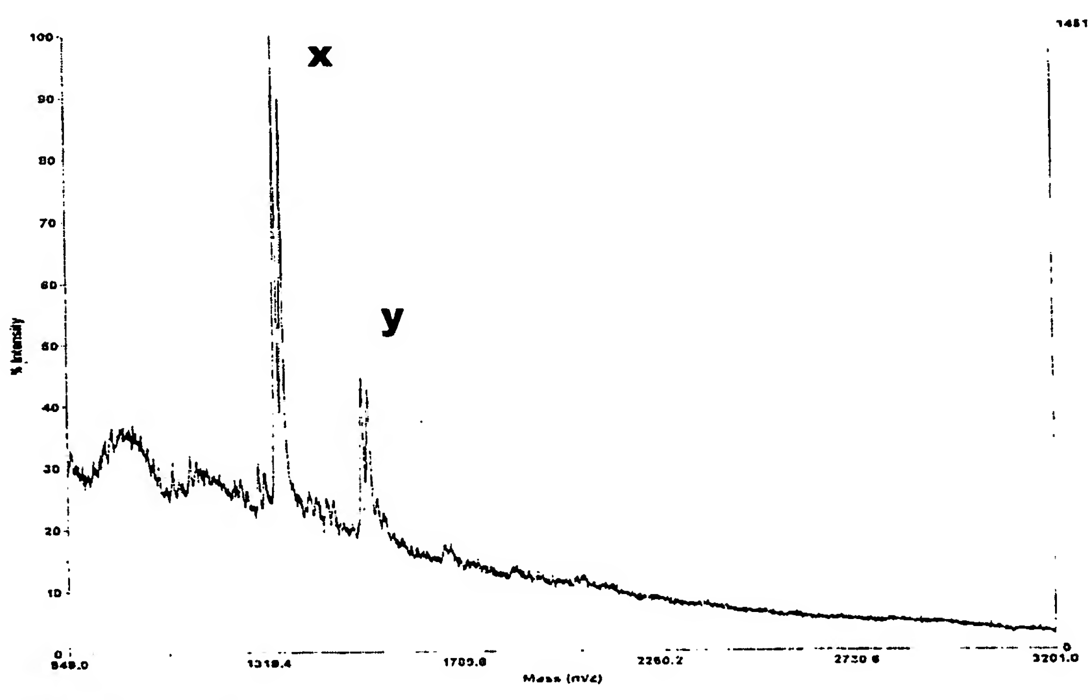

x
2493.0
% Intensity
100
90
80
70
60
50
40
30
20
10
0
849.0
1319.4
1789.8
2260.2
2730.6
3201.0
Mass (m/z)

Fig. 32


x
y
1451
% Intensity
100
90
80
70
60
50
40
30
20
10
0
849.0
1319.4
1789.8
2260.2
2730.6
3201.0
Mass (m/z)

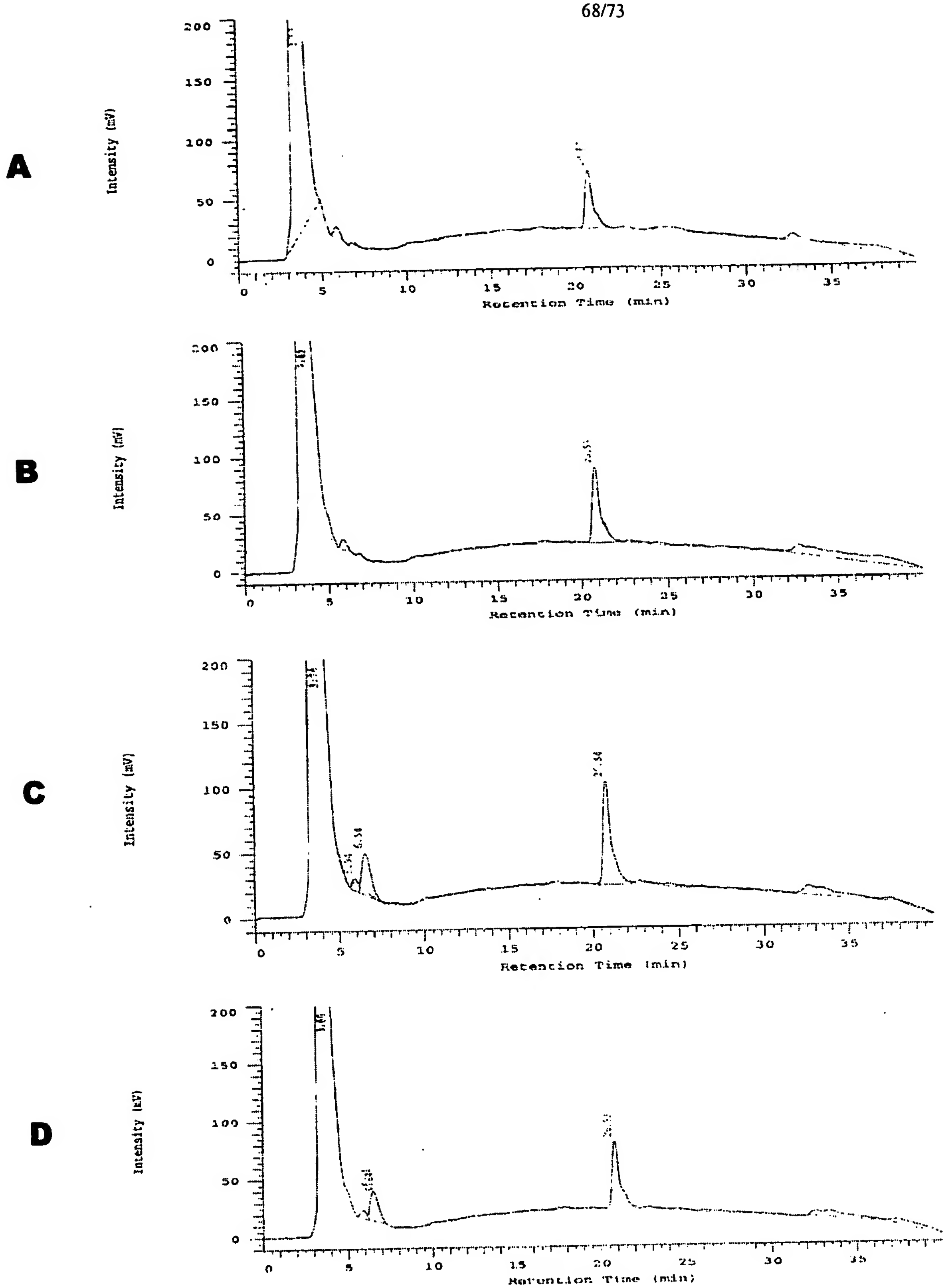

A
B
C
D
Intensity (mV)
Retention Time (min)
0
50
100
150
200
5
10
15
20
25
30
35

Fig. 33

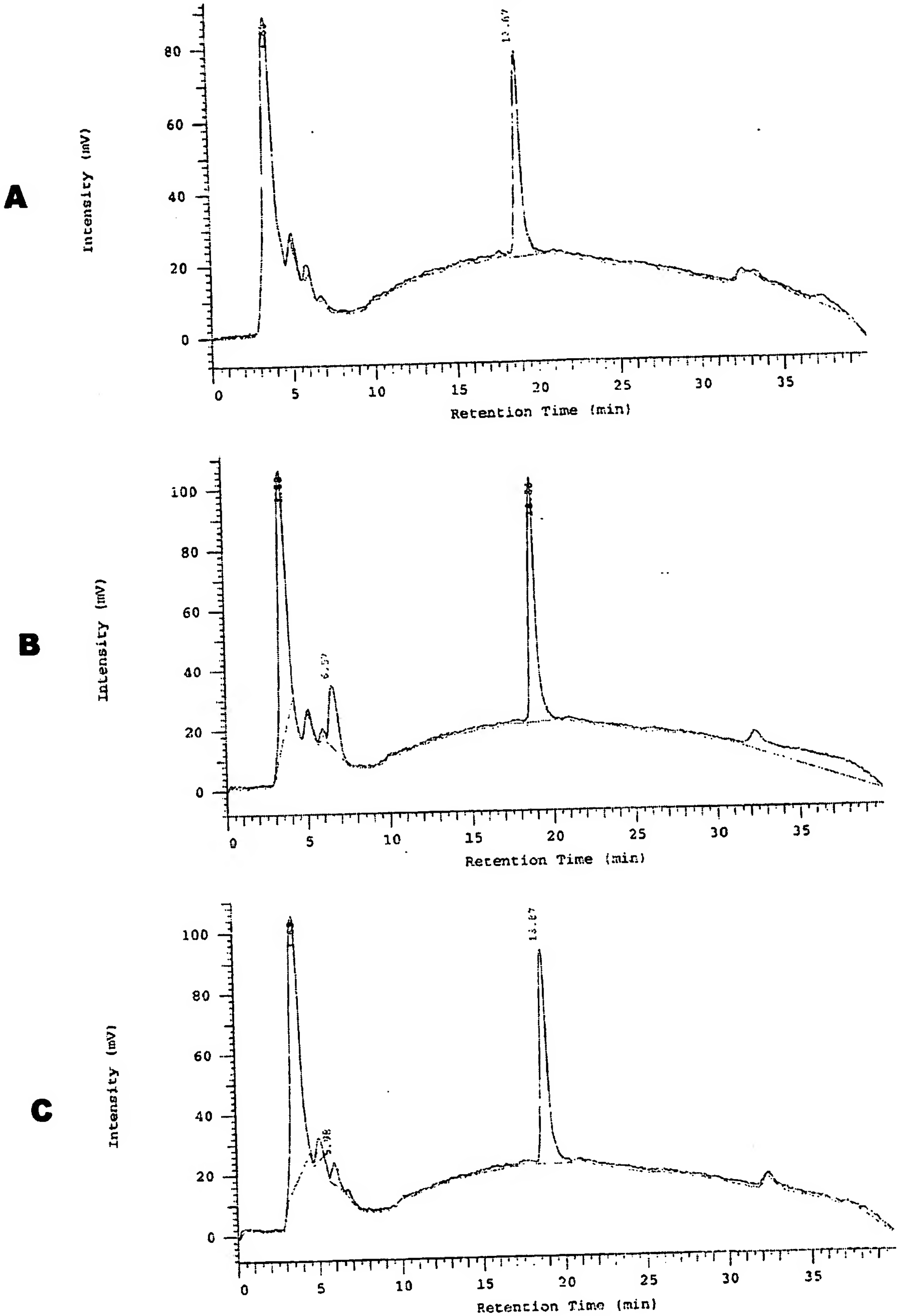

A
B
C
Intensity (mV)
Retention Time (min)
0
5
10
15
20
25
30
35
20
40
60
80
100
6.57

Fig. 34

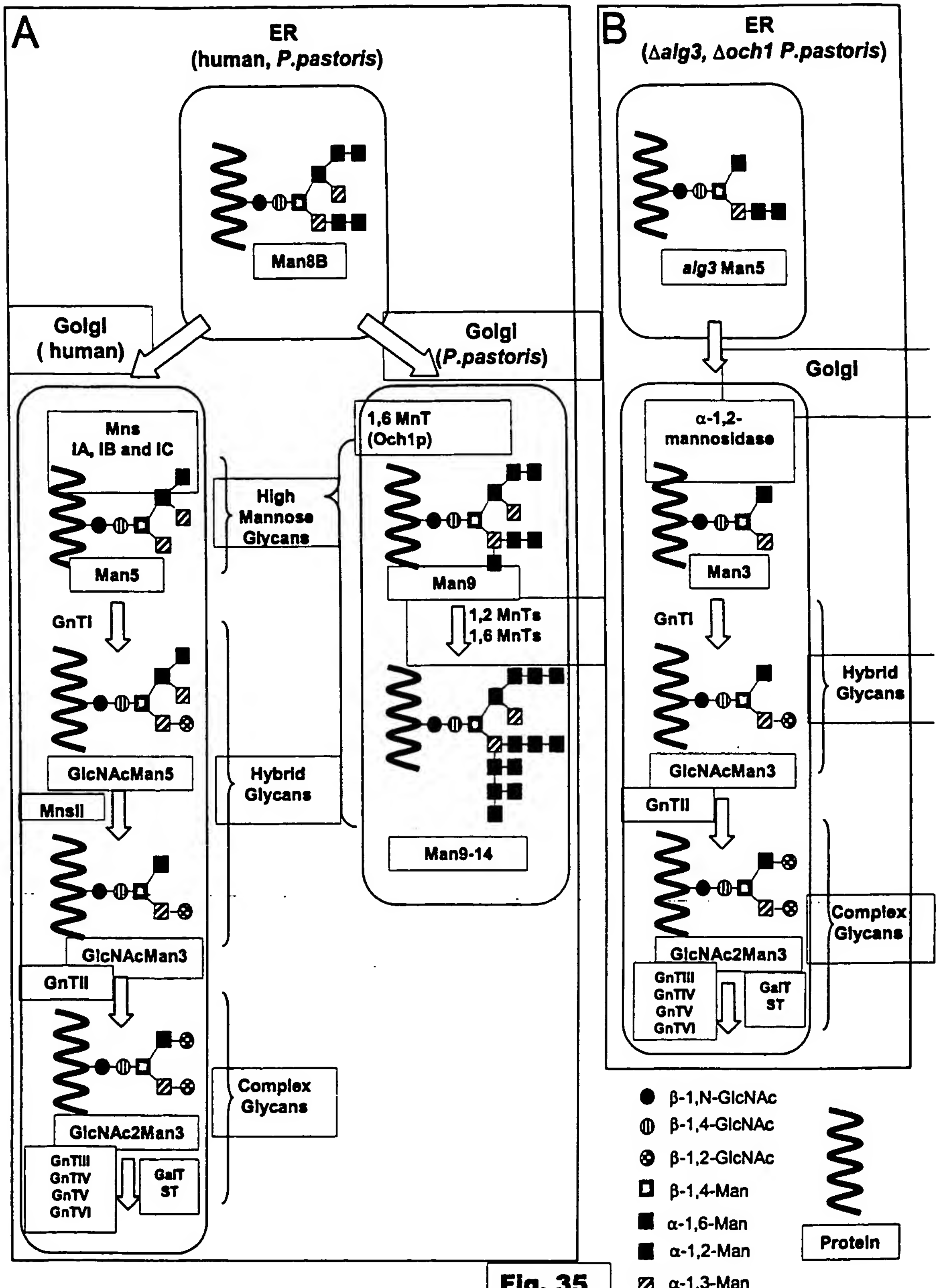

A
ER
(human, P.pastoris)
Man8B
Golgi
(human)
Golgi
(P.pastoris)
Mns
IA, IB and IC
Man5
High
Mannose
Glycans
GnTI
GlcNAcMan5
Hybrid
Glycans
MnsII
GlcNAcMan3
GnTII
Complex
Glycans
GlcNAc2Man3
GnTIII
GnTIV
GnTV
GnTVI
GalT
ST
1,6 MnT
(Och1p)
Man9
1,2 MnTs
1,6 MnTs
Man9-14
B
ER
(Δalg3, Δoch1 P.pastoris)
alg3 Man5
Golgi
α-1,2-
mannosidase
Man3
GnTI
Hybrid
Glycans
GlcNAcMan3
GnTII
Complex
Glycans
GlcNAc2Man3
GnTIII
GnTIV
GnTV
GnTVI
GalT
ST
β-1,N-GlcNAc
β-1,4-GlcNAc
β-1,2-GlcNAc
β-1,4-Man
α-1,6-Man
α-1,2-Man
α-1,3-Man
Protein

Fig. 35

Fig. 36

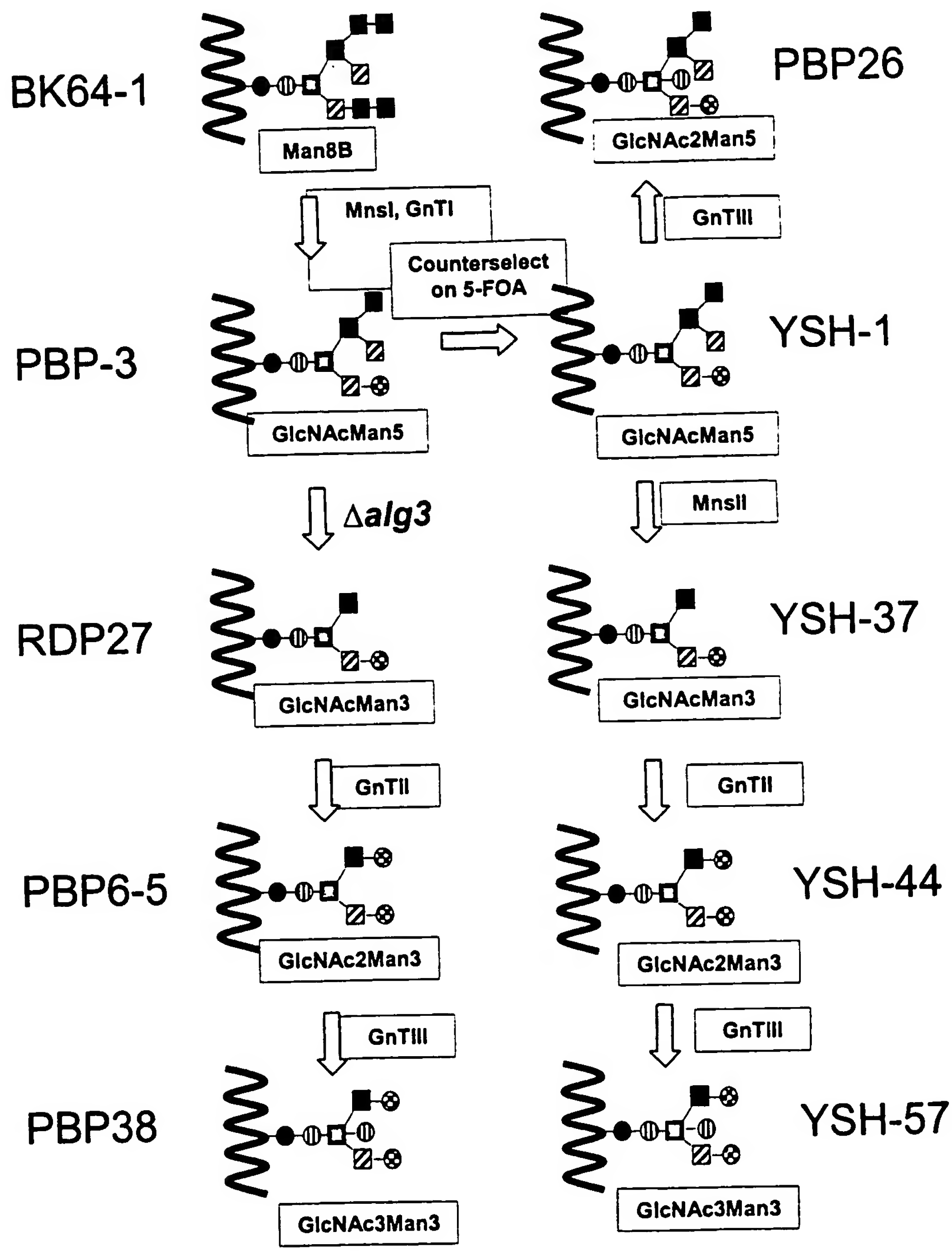

BK64-1
Man8B
PBP26
GlcNAc2Man5
MnsI, GnTI
GnTIII
Counterselect on 5-FOA
PBP-3
GlcNAcMan5
YSH-1
GlcNAcMan5
Δalg3
MnsII
RDP27
GlcNAcMan3
YSH-37
GlcNAcMan3
GnTII
GnTII
PBP6-5
GlcNAc2Man3
YSH-44
GlcNAc2Man3
GnTIII
GnTIII
PBP38
GlcNAc3Man3
YSH-57
GlcNAc3Man3

Fig. 37

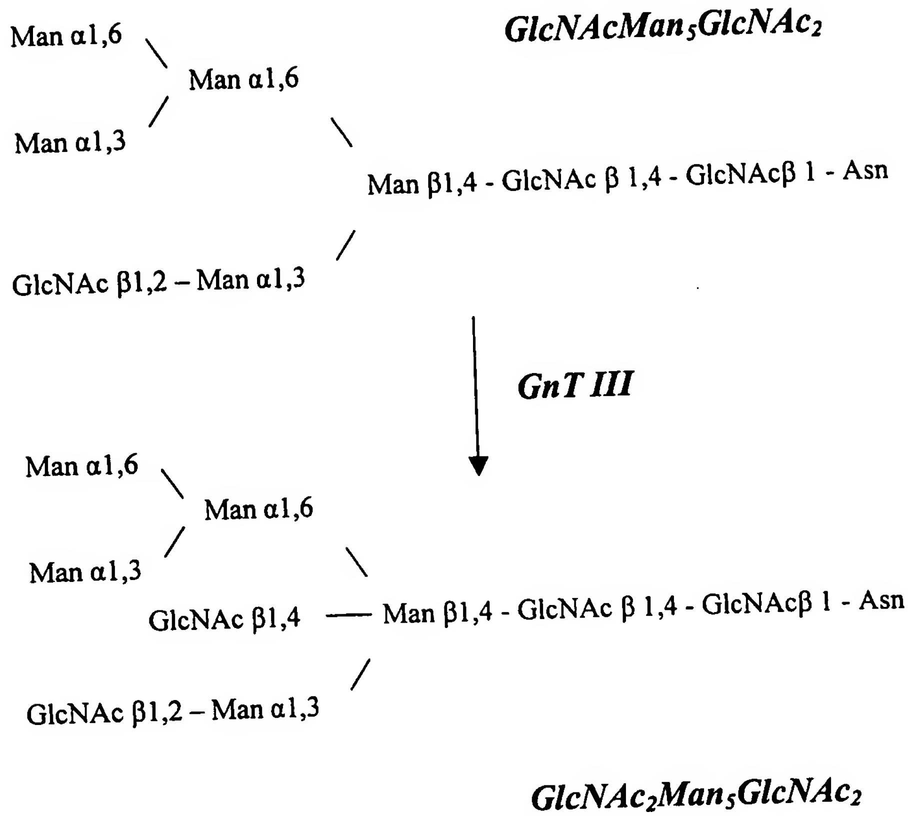
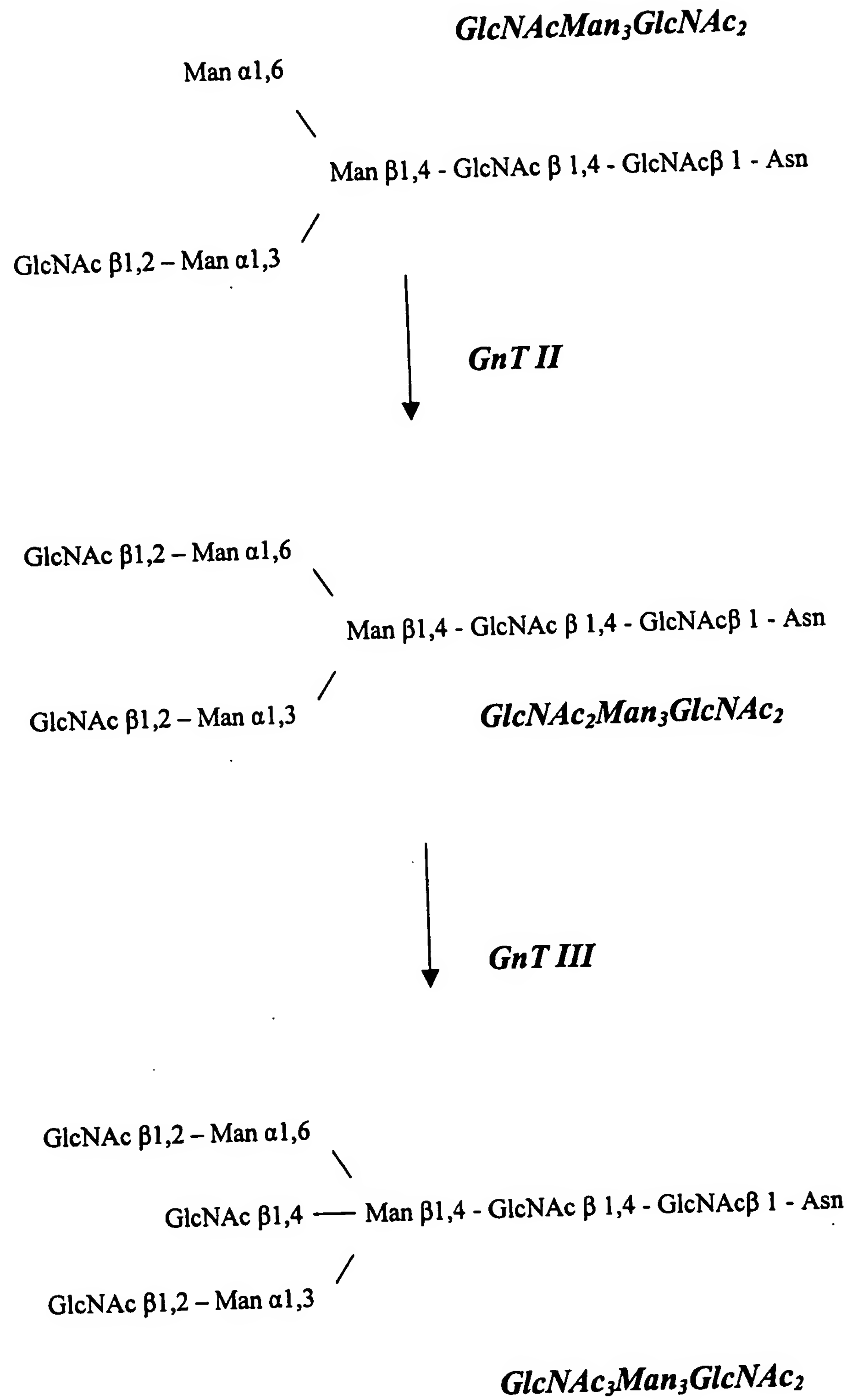

GlcNAcMan5GlcNAc2
Man α1,6
Man α1,6
Man α1,3
Man β1,4 - GlcNAc β 1,4 - GlcNAcβ 1 - Asn
GlcNAc β1,2 – Man α1,3
GnT III
Man α1,6
Man α1,6
Man α1,3
GlcNAc β1,4
Man β1,4 - GlcNAc β 1,4 - GlcNAcβ 1 - Asn
GlcNAc β1,2 – Man α1,3
GlcNAc2Man5GlcNAc2

Fig. 38


GlcNAcMan3GlcNAc2
Man α1,6
Man β1,4 - GlcNAc β 1,4 - GlcNAcβ 1 - Asn
GlcNAc β1,2 – Man α1,3
GnT II
GlcNAc β1,2 – Man α1,6
Man β1,4 - GlcNAc β 1,4 - GlcNAcβ 1 - Asn
GlcNAc β1,2 – Man α1,3
GlcNAc2Man3GlcNAc2
GnT III
GlcNAc β1,2 – Man α1,6
GlcNAc β1,4 — Man β1,4 - GlcNAc β 1,4 - GlcNAcβ 1 - Asn
GlcNAc β1,2 – Man α1,3
GlcNAc3Man3GlcNAc2